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Mgr. Martin Čertner (roz. Hanzl)

Mikroevoluční procesy v cytotypově smíšených populacích rostlin
Microevolutionary processes in mixed-ploidy populations of plants

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Školitel / Supervisor: RNDr. Filip Kolář, Ph.D.

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Prohlášení autora / Author's declaration

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I hereby declare that I made this thesis independently, using only the mentioned refereces. I did not submit this thesis nor its part for any other degree or diploma.

V Praze dne 1. 10. 2017

Martin Čertner

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Abstract

Polyploidization (whole-genome duplication) is widely considered one of the most important evolutionary forces driving the diversification of flowering plants. Polyploids tend to originate recurrently and many plant species retain individuals of two or more different ploidy levels in certain parts of their distributional range of even within their populations. The main aim of this thesis was to address the understudied aspects of polyploid speciation by employing new, convenient methods and/or studying plant model systems with unique features. Difference in monoploid genome size of *Tripleurospermum inodorum* (Asteraceae) cytotypes provided a unique opportunity for addressing the rate of spontaneous polyploidization in natural populations by enabling the easy distinction of neopolyploid mutants from long-established polyploids in routine flow-cytometric analyses. Repeated ploidy screening in mixed-ploidy populations of annual *T. inodorum* have been, to our knowledge, the very first attempt to document temporal changes in cytotype composition *in situ*. In spite of considerable between-year oscillations in cytotype frequencies, both diploids and tetraploids usually persisted locally for several consecutive years. The common incidence of such ploidy mixtures along with a partial fertility of triploid hybrids then likely result in gene flow between coexisting di- and tetraploids. A new insight into the direct phenotypic and ecological consequences of genome doubling was accomplished in a unique primary contact zone of diploids and their recent autotetraploid derivates in *Knautia serpentinicola* (Caprifoliaceae). Not only did the tetraploids attain higher values of both aboveground and belowground biomass compared to their diploid progenitors, when provided enough nutrients, they were also more successful in tolerating interspecific competition, which might have been the key to their successful establishment. When evaluating the importance of habitat differentiation for cytotype coexistence in both *T. inodorum* and *K. serpentinicola*, we demonstrated that independent replicates and/or across-scale comparisons may significantly improve the performance of ecological niche assessments. In the contact zones of both model species, at regional and finer scales, non-adaptive processes (e.g. non-random migration patterns, the founder effect) were more important determinants of cytotype distribution patterns than environmental heterogeneity. Finally, we reviewed the available literature on intraspecific ploidy variation, highlighting unexpectedly high cytotype diversity and a frequent ploidy coexistence in the cytologically intensely surveyed species. Numerous mechanisms facilitating the ploidy coexistence have been suggested, however, only a few of these were sufficiently explored. The roles of a spatial segregation of cytotypes and asexual reproduction were emphasized in our survey.

Key words: cytotype coexistence, ecological niche differentiation, genome doubling, minority cytotype exclusion, neopolyploid establishment, polyploid speciation

Abstrakt

Polyploidizace (duplikace genomu) je obecně přijímána jako jeden z nejvýznamnějších procesů zodpovědných za evoluční diverzifikaci krytosemenných rostlin. Ke vzniku polyploidů dochází opakovaně a řada rostlinných druhů si udržuje dva či více různých cytotypů v určitých částech svého areálu nebo dokonce ve svých populacích. Cílem této práce bylo využít příhodné metodické postupy a modelové rostliny s unikátními vlastnostmi k cílenému studiu doposud neobjasněných aspektů polyploidní speciace. Rozdíly v monoploidní velikosti genomu mezi cytotypy *Tripleurospermum inodorum* (Asteraceae) byly unikátní příležitostí ke studiu frekvence vzniku nových polyploidů v přirozených populacích, neboť umožňují snadné odlišení polyploidních mutantů od již etablovaných polyploidů v rutinních cytometrických analýzách. Cytotypový průzkum opakovaný ve smíšených populacích jednoletého druhu *T. inodorum* byl patrně úplně prvním pokusem zdokumentovat časový vývoj cytotypového složení v přírodních populacích. Navzdory značným meziročním fluktuacím ve frekvenci cytotypů, jak diploidi tak tetraploidi byli schopni v populacích vytrvat po několik následných let. Častý výskyt takových smíšených populací pak, spolu s částečnou fertilitou triploidních hybridů, přispívají ke genovému toku mezi koexistujícími di- a tetraploidy. Unikátní primární kontaktní zóna mezi diploidy a z nich nedávno vzniklými autotetraploidy u *Knautia serpentinicola* (Caprifoliaceae) umožnila nahlédnout do problematiky přímých fenotypových a ekologických důsledků duplikace genomu. Tetraploidi nejen že dosahovali větších hodnot nadzemní i podzemní biomasy oproti svým diploidním předkům, ale v případě dostatku živin byli zároveň i úspěšnější v tolerování mezidruhové kompetice, což mohlo sehrát klíčovou roli v jejich úspěšném uchycení na lokalitě. Během hodnocení rozdílů v ekologických nárocích a míry jejich vlivu na koexistenci cytotypů jsme u *T. inodorum* i *K. serpentinicola* demonstrovali, že nezávislá opakování i srovnání napříč škálami mohou značně zvýšit přesnost ekologických porovnání cytotypů. V kontaktních zónách obou modelových druhů bylo patrné, že na menších prostorových škálách je distribuce cytotypů ovlivněna více neadaptivními procesy (např. nenáhodnost migrace, efekt zakladatele) než ekologickou různorodostí prostředí. Práce byla doplněna rešerší literatury zaměřené na téma vnitrodruhové cytotypové variability, která odhalila překvapivou diverzitu cytotypů a jejich častou koexistenci u většiny excerpovaných druhů. Přestože byla v literatuře navržena celá řada mechanismů přispívajících ke koexistenci cytotypů, jen malá část z nich byla dostatečně empiricky podložena. Ke společnému výskytu různých cytotypů u řady druhů významně přispívá jejich prostorová segregace a převládající nepohlavní rozmnožování.

Klíčová slova: koexistence cytotypů, rozrůznění ekologických nik, duplikace genomu, nevýhoda malých čísel, uchycování neopolyploidů, polyploidní speciace

List of included papers

I. **Hanzl, M.***, Kolář, F., Nováková, D. & Suda, J. 2014. Nonadaptive processes governing early stages of polyploid evolution: Insights from a primary contact zone of relict serpentine *Knautia arvensis* (Caprifoliaceae). American Journal of Botany, 101(6): 935-945.

II. **Čertner, M.**, Sudová, R., Weiser, M., Suda, J. & Kolář, F. Phenotypic consequences of genome doubling in stressful environments: Serpentine bedrock shapes the diploid – tetraploid coexistence in *Knautia serpentinicola* (Caprifoliaceae). Manuscript.

III. **Čertner, M.**, Fenclová, E., Kúr, P., Kolář, F., Koutecký, P., Krahulcová, A. & Suda, J. 2017. Evolutionary dynamics of mixed-ploidy populations in an annual herb: dispersal, local persistence and recurrent origins of polyploids. Annals of Botany, 120(2): 303-315.

IV. **Čertner, M.**, Kúr, P., Kolář, F. & Suda, J. Climatic conditions and human activities shape diploid – tetraploid coexistence at different spatial scales in the common weed *Tripleurospermum inodorum* (Asteraceae). Manuscript.

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* The first paper was published under my birth name.

Author contribution statement

I declare that I have substantially contributed to all papers included in the thesis. Specifically, my contributions to particular papers are as follows:

I. Hanzl, M., Kolář, F., Nováková, D. & Suda, J. 2014. Nonadaptive processes governing early stages of polyploid evolution: Insights from a primary contact zone of relict serpentine *Knautia arvensis* (Caprifoliaceae).

Study design, field sampling, data analysis, and manuscript preparation – **total contribution 80%**

II. Čertner, M., Sudová, R., Weiser, M., Suda, J. & Kolář, F. Phenotypic consequences of genome doubling in stressful environments: Serpentine bedrock shapes the diploid – tetraploid coexistence in *Knautia serpentinicola* (Caprifoliaceae).

Study design, experimental cultivation, data analysis, and manuscript preparation – **total contribution 90%**

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Study design, field sampling, data analysis, and manuscript preparation – **total contribution 70%**

IV. Čertner, M., Kúr, P., Kolář, F. & Suda, J. Climatic conditions and human activities shape diploid – tetraploid coexistence at different spatial scales in the common weed *Tripleurospermum inodorum* (Asteraceae).

Study design, field sampling, data analysis, and manuscript preparation – **total contribution 80%**

V. Kolář, F., Čertner, M., Suda, J., Schönswetter, P. and Husband, B. 2017. Mixed-ploidy species: Progress and opportunities in polyploid research.

Literature survey, data collection, and manuscript preparation – **total contribution 40%**

Part A – General chapters

1. Polyploidy

Polyploidy, the possession of more than two complete sets of chromosomes in the nucleus, is widely considered one of the most important evolutionary forces driving the diversification of flowering plants (Soltis et al., 2009; Weiss-Schneeweiss et al., 2013). Recent studies suggest that all angiosperms have undergone one or more polyploidization events in their evolutionary history (Wendel et al., 2016). The evolutionary significance of polyploidy stems mainly from an instantaneous reproductive isolation from diploid progenitors, which makes it an efficient mechanism of sympatric speciation (Coyne and Orr, 2004; Wood et al., 2009), and from the fact that polyploidization often brings genetic and phenotypic novelty (Levin, 1983; Flagel and Wendel, 2009; Hegarty et al., 2013). Even though polyploidy has fascinated botanists ever since its discovery at the beginning of 20th century, the study of polyploids have enjoyed a renaissance over the past decade (Barker, Husband, et al., 2016), which was driven mainly by advances in cytogenetics (e.g. the frequent employment of flow-cytometric ploidy screening; Doležel et al., 2007) and recently in genomics (e.g. the massively parallel sequencing; Wendel et al., 2016).

The vast majority of polyploid mutants emerge in populations of their diploid progenitors due to meiotic errors, as a result of crosses involving gametes with somatic chromosome number (i.e., unreduced gametes; Harlan and De Wet, 1975; Ramsey and Schemske, 1998). Gametic non-reduction is generally a rare process, with a mean occurrence of 0.56% across non-hybrid angiosperms (Ramsey and Schemske, 1998), however, substantially increased rates of unreduced-gamete production were documented for some mutants in natural populations (Ramsey, 2007) and for plants growing under stressful conditions (reviewed in Levin, 2002). Tetraploid mutants may not only result from a fusion of two unreduced gametes of diploids but, alternatively, a single unreduced gamete may produce a triploid intermediate that can give rise to tetraploid offspring in crosses with diploids and/or other triploids (i.e., the triploid bridge mechanism). The latter scenario assumes a certain level of triploid viability and fertility, which has been met in several mixed-ploidy species (Husband, 2004).

Two major types of polyploidy are usually discerned (Grant, 1981): intraspecific polyploidy, originating within populations of a single species (i.e. autopolyploidy) and interspecific polyploidy, when genome doubling is preceded or followed by interspecific hybridization (i.e. allopolyploidy). Based on genetic and cytogenetic criteria, the presence of multivalents at meiosis and polysomic inheritance is typical for autopolyploids, whereas the allopolyploids are characterized by predominant bivalent formation and disomic interitance (Stebbins, 1947). However, rather than two exclusive options, auto- and allopolyploidy represent two extremes of a continuum (Levin, 2002). A long-lasting argument on the relative incidence of auto- vs. allopolyploidy (Stebbins, 1947; Lewis, 1980; Grant, 1981; Soltis et al., 2007) have recently resulted in a literature survey of the available evidence (Barker, Arrigo, et al., 2016). The rates of autopolyploid formation seem to markedly exceed those of allopolyploid origin (Ramsey and Schemske, 1998) but, likely due to much stronger constraints on autopolyploid establishment, the both types of polyploids are more-or-less equally represented in nature (Barker, Arrigo, et al., 2016). Even though in many plant taxa the mode of polyploid origin is still unresolved, in this thesis I am primarily focusing on the autopolyploidy.

2. Cytotype coexistence in plant populations

Many plant species retain individuals of two or more different ploidy levels in certain parts of their distributional range (e.g., Kliber and Eckert, 2005; Castro et al., 2012; Godsoe et al., 2013; Kolář et al., 2013) or even within their populations (e.g., Kao, 2007; Sonnleitner et al., 2010; Šafářová and Duchoslav, 2010; Trávníček, Dočkalová, et al., 2011). Local coexistence of different cytotypes may arise either from an *in-situ* polyploidization event, resulting in the sympatric occurrence of genetically close di- and polyploids (i.e., primary contact) or through cytotype immigration into a population of another ploidy (i.e., secondary contact; Petit et al., 1999). Mixed-ploidy populations serve as a convenient microcosm for the study of factors affecting the origin and establishment of polyploids (Husband, 2004). In addition, they offer an opportunity to investigate the evolution of already established cytotypes that may be further subjected to inter-ploidy gene flow (e.g., Arnold et al., 2015; Hülber et al., 2015) or shaped by the selection for more efficient prezygotic breeding barriers (Van Dijk and Bijlsma, 1994; Nuismer and Cunningham, 2005). Given that polyploidization is a major mechanism of sympatric speciation, such studies may also address more general questions regarding the origin of new plant species.

2.1 Minority cytotype exclusion principle

According to theoretical predictions, a coexistence of two or more cytotypes within a population should be unstable, representing only a transitory state before local fixation of one of the cytotypes (Levin, 1975; Fowler and Levin, 1984). This stems from the fact that pollen exchange among plants differing in ploidy level results in ineffective matings, because the progeny of intermediate, often odd-numbered ploidy level suffers from lower viability and fertility (Ramsey and Schemske, 1998). The fitness of a minority cytotype will thus be reduced, not because it is less well adapted, but because it is less common, has fewer potential mates, and experiences more ineffective matings. As a result of frequency-dependent selection acting on cytotypes in mixed-ploidy populations, the populations will ultimately become fixed for one (presumably the locally most abundant) cytotype in a process referred to by Levin (1975) as "minority cytotype exclusion". Such frequency-dependent mating disadvantage has been repeatedly observed in both experimental and natural mixed-ploidy populations (Hagberg and Ellerström, 1959; Maceira et al., 1993; Husband, 2000; Baack, 2005a; Mráz et al., 2012). Aside from the reproductive interference, coexisting cytotypes also face direct resource competition within mixed-ploidy populations. Nonetheless, this aspect of cytotype coexistence has received much less attention (Collins et al., 2011).

2.2 Overcoming the frequency-dependent mating disadvantage

Interestingly, there are several factors in natural mixed-ploidy plant populations that might compensate the minority cytotype disadvantage. Their incorporation into theoretical models either slowed down the fixation of one of the cytotypes or, under specific combinations of parameter values, reached the coexistence of cytotypes in a more-or-less stable equilibrium (Rodríguez, 1996; Husband, 2004; Li et al., 2004; Yamauchi et al., 2004; Baack, 2005b; Rausch and Morgan, 2005; Oswald and Nuismer, 2011; Suda and Herben, 2013).

What is the nature of these stabilizing forces? Firstly, it is only the output of sexual reproduction that is frequency dependent in mixed-ploidy populations. If the species relies at least occasionally on some way of vegetative propagation (e.g. production of bulbils and rhizome buds in *Butomus umbellatus*, Hroudová and Zákravský, 1993; spreading by tillers in *Dactylis glomerata*, Bretagnolle and Thompson, 2001), the decline of a minority cytotype may be counteracted. A specific case of vegetative propagation known to occur nearly exclusively among polyploids is agamospermy (= apomixis *s. str.*; Bicknell and Koltunow, 2004), i.e. a process when clonal progeny is produced by seeds. In the populations of *Arnica cordifolia*, where agamospermy is the prevailing mode of reproduction, any association between the frequency of a cytotype in mixed-ploidy population and its seed set was lacking (Kao, 2007). Another mode of reproduction that is particularly common among polyploids is autogamy (Barringer, 2007). Indeed, self-pollination is an effective way of reproductive assurance, especially for rare minority cytotypes, unless hampered by strong inbreeding depression (Husband and Schemske, 1997; Rausch and Morgan, 2005; Husband et al., 2008).

Secondly, the model predictions suggesting rapid fixation of one of the cytotypes in initially mixed-ploidy populations are only valid under the assumption of random mating, which might be seriously violated in natural populations. Assortative (non-random) mating between the cytotypes either may be a consequence of ploidy-specific differences in their adaptive traits or it may stem from non-adaptive explanations, such as inherent plant traits and/or chance events. Among the adaptive scenarios, shifts in phenology of sympatric cytotypes that reduce the overlap in their flowering times are frequently reported in mixed-ploidy species (e.g., *Arrhenatherum elatius*, Petit et al., 1997; *Heuchera grossulariifolia*, Nuismer and Cunningham, 2005). Interestingly, the cytotypes frequently differ in floral display and/or floral scent (Levin, 2002; Jersáková et al., 2010), and these visual and olfactory cues may in some cases affect pollinator behavior and increase the rate of assortative mating, albeit this was only rarely documented (Segraves and Thompson, 1999; Kennedy et al., 2006). A fine-scale spatial clustering of cytotypes in mixed-ploidy populations was reported from most of the investigated species (e.g., *Allium oleraceum*, Šafářová and Duchoslav, 2010; *Andropogon gerardii*, Keeler, 2004; *Gymnadenia conopsea*, Trávníček, Kubátová, et al., 2011; *Larrea tridentata*, Laport and Ramsey, 2015) and has been shown to lead to assortative mating (Baack, 2005b), albeit the reasons behind cytotype segregation may differ from case to case. For example, the cytotype distribution patterns may be correlated with habitat heterogeneity, and follow ploidy-specific differences in survival and/or fitness under certain environmental conditions (e.g., *Dactylis glomerata*, Lumaret et al., 1987; *Achillea borealis*, Ramsey, 2011). Distinct colonization histories of particular cytotypes at a given site may also cause their spatial clustering, e.g. if one of the cytotypes is being unintentionally introduced into already established populations of the other cytotype by human activities, as was suggested for *Centaurea stoebe* (Mráz et al., 2012; Otisková et al., 2014) and *Taraxacum* sect. *Ruderalia* (Meirmans et al., 1999). However, the fine-scale clustering of cytotypes may also result from limited seed dispersal and/or effective spread by means of vegetative reproduction (Baack, 2005b); both of these are inherent traits of many plant species, irrespective of their ploidy level.

Thirdly, even if a species relies solely on sexual reproduction and assortative mating proves inefficient in preventing across-ploidy pollen transfers, several other factors may affect the chances of a minority cytotype. For instance, fertilization success of the cytotypes may strongly differ if one of the cytotypes is superior in pollen competition (tetraploids of *Chamerion angustifolium* and *Hieracium echioides*; Husband et al., 2002; Peckert and Chrtek, 2006), albeit this is probably not a general situation in all mixed-ploidy systems (see Baack, 2005a). The chances of preserving a minority cytotype can be enhanced if the cytotype originates recurrently (e.g., due to increased production of unreduced gametes, from across-ploidy hybridizations; Peckert and Chrtek, 2006; Ramsey, 2007) or as a result of frequent immigration of the cytotype from surrounding populations of a contact zone (Levin, 1975). Moreover, the lower seed set of minority cytotypes can be at least partially compensated by their superior competitive abilities, which may stem from their higher overall vigour (Keeler and Davis, 1999), different life history strategies (Hahn et al., 2012), and/or more balanced interactions with other groups of organisms (e.g. herbivores, parasites, mutualists; Nuismer and Thompson, 2001; Arvanitis et al., 2008; Sudová et al., 2014). The frequency-dependent selection driving the less common cytotype to local extinction may also be disrupted if the overall population size oscillates in time or as a result of recurring disturbances (Rausch and Morgan, 2005; Halverson et al., 2008). Finally, even the minority cytotype exclusion principle (Levin, 1975), that is considered to be the main obstacle to cytotype coexistence, may in some cases enhance the stability of parapatric cytotype contacts by limiting the area in which the cytotypes intermingle (i.e. forming a ”tension zone”; Van Dijk et al., 1992; Baack, 2004).

3. The understudied aspects of cytotype coexistence

Here I will focus on several aspects of cytotype coexistence that either have not received enough attention so far or where some pitfalls could complicate drawing general conclusions from the available data. Following the introduction of these topics, I will then postulate specific objectives of the presented thesis (see Chapter 4).

3.1 The success of polyploid establishment

The establishment of polyploid mutants in diploid populations generally follows the same rules as local coexistence of already established cytotypes in contact zones (Chapter 2). Success of novel polyploids thus depends primarily on the strength of frequency-dependent selection against the minority cytotype (Levin, 1975; Husband, 2000). However, the establishment of polyploid mutants has also several specifics. Firstly, the rate of their origin is generally low (Harlan and De Wet, 1975; Ramsey and Schemske, 1998), so polyploid mutants occur solitarily and are in profound minority. Secondly, new polyploids originate from diploid or lower polyploid parents; they are thus typically surrounded by their progenitors, which leads to stronger inter-ploidy interactions. Thirdly and most importantly, first generations of polyploid mutants possess only a set of inherent traits of a particular species (e.g. the capacity of clonal reproduction, limited seed dispersal) and innovations directly resulting from the polyploidization event (which may not always be beneficial – e.g. meiotic irregularities reducing the fertility of autopolyploids; Yant et al., 2013).

However, our understanding of the mechanisms of successful polyploid establishment is mostly based on studying the outputs of this process, i.e., by comparing long-established polyploids with their diploid relatives. Phenotypes of such "old" polyploids, however, could have been significantly shaped by local adaptation in allopatry and/or genetic drift (Petit et al., 1999). Since the direct effects of genome doubling may be confounded with other changes gradually accumulating after the polyploidization event, the phenotypic and ecological consequences of genome duplication might be overestimated (Ramsey, 2011). There are principally three ways of disentangling the direct effects of genome duplication from the long-term differentiation of cytotypes, all based on the presumption of comparing individuals with common genetic background that differ in ploidy level. The first and by far the most commonly used technique is production of synthetic polyploids, where the genome duplication is induced by treating the progenitor's tissue with a chemical disrupting mitosis, such as colchicine (Husband et al., 2008; Oswald and Nuismer, 2011; Hegarty et al., 2013). While the use of synthetic polyploids is a method of general applicability, not limited to a handful of suitable plant model systems, some authors have argued that not just genome doubling but also the shock induced by application of colchicine may shape the phenotypes of synthetic polyploids (Ramsey and Schemske, 2002). The second method is based on flow-cytometric screening of first-generation polyploid mutants either from natural populations of its lower-ploidy progenitor (Ramsey, 2011) or among progeny produced in artificial crosses among diploid parents where unreduced gametes were involved (Bretagnolle and Lumaret, 1995). Here the main advantage is the possibility of studying the first-generation polyploids that originated spontaneously, albeit the rare occurrence of natural neopolyploids may lead to extremely laborious gain of the experimental material.

However, all these methods share a common drawback – the first-generation polyploid offspring results from an arbitrary selection made by the researchers, which can significantly impact the range of phenotypic variation among new polyploids. A convenient alternative to these approaches is comparing biological properties of diploids and their sympatric, recently derived autopolyploids that still coexist in natural populations, so-called primary contact zones (e.g., Bretagnolle and Thompson, 2001; Šingliarová et al., 2011; Kolář et al., 2012). The main strengths of this approach are 1) the opportunity to compare performance of the two cytotypes under natural conditions *in situ* and 2) capturing only "biologically-relevant" variation, as maladapted polyploid mutants are not retained in natural populations. So far, however, only a handful of studies addressed phenotypic consequences of genome doubling in a primary contact zone (in a single species, *Dactylis glomerata*; Lumaret et al., 1987; Maceira et al., 1993).

Based on data collected using the three above described approaches, there is only a limited set of phenotypic changes that seem to regularly accompany genome duplication events. Most of them are consequences of the so-called "gigas effect" (Stebbins, 1971), an increase in polyploid cell size that often translates into increased organ size and greater plant biomass. Polyploid mutants tend to have larger stomata, pollen grains, leaves, and greater total biomass (e.g., Maceira et al., 1993; Bretagnolle and Lumaret, 1995; Oswald and Nuismer, 2011; Ramsey, 2011; Hegarty et al., 2013). Since these phenotypic changes are only rarely substantial, it is likely that the role of initial fitness advantage experienced by polyploid mutants during their establishment has been overrated by studies focusing on secondary contact zones. On the other hand, the chances of successful polyploid establishment may be elevated also by traits inherent to many plant species (e.g. the capacity of clonal reproduction, plant longevity, and limited seed dispersal; Yamauchi et al., 2004; Baack, 2005b). Moreover, even minimal chances of overcoming the minority cytotype exclusion may not constrain the polyploid establishment in a long time frame, because the polyploid mutants originate recurrently in diploid populations (Segraves et al., 1999; Soltis and Soltis, 1999; Brochmann et al., 2004; Thompson and Whitton, 2006) and occasional stochastic events (e.g., disturbances, oscillations in abundance of the progenitor's population) may act in favour of the polyploid. Such non-adaptive scenarios have not received enough attention so far (but see Lewis, 1967; Van Dijk and Bakx-Schotman, 1997).

3.2 Environmental drivers of cytotype coexistence

Since the early studies, contrasting ecogeographical distributions of closely related diploids and polyploids have been frequently reported (reviewed in Ehrendorfer, 1980; Levin, 2002). Based on that, a great emphasis was taken on the condition of habitat differentiation of sympatric cytotypes (Ramsey and Ramsey, 2014), i.e., a situation when ploidy-specific differences in survival and/or fitness under certain environmental conditions prevent each cytotype from successfully invading the habitat of the other cytotype. Theoretical models have shown that distinct habitat preferences of cytotypes could lead to their spatial segregation in environmentally heterogeneous landscapes and thus allow both polyploid establishment and subsequent cytotype coexistence (Fowler and Levin, 1984; Rodríguez, 1996). The habitat segregation of cytotypes was indeed repeatedly documented in nature, either as a mechanism allowing the establishment of young polyploid derivatives in populations of their lower-ploid progenitors (Lumaret et al., 1987; Ramsey, 2011) or, as a way of facilitating the coexistence of already established cytotypes encountering each other in secondary contact zones (e.g., Manzaneda et al., 2012; Laport et al., 2013; Sonnleitner et al., 2016). On the other hand, the lack of habitat segregation of cytotypes was observed in many other studies (e.g., Baack and Stanton, 2005; Buggs and Pannell, 2007) and no consistent trends in habitat preferences (in this case climatic niche) were detected in a comprehensive set of 20 conspecific diploid – young polyploid comparisons (Glennon et al., 2014).

Although these inconsistencies might simply suggest, that ecological segregation of cytotypes is rather an occasional scenario than the rule, we still have no estimates of the actual rate at which this process is involved across species due to several pitfalls. Firstly, different authors use different methods for identifying the cases of ecological segregation of cytotypes such as assessments of (macro-)climatic conditions, habitat-type preferences, vegetation composition, and local environmental parameters (either directly measured in the field or reconstructed from the species composition using ecological indicator values). Use of different methods thus hampers across-species generalizations. Unfortunately, comprehensive studies employing more than one of these approaches are still rare (Treier et al., 2009; Laport et al., 2013; Kirchheimer et al., 2016). Secondly, each method is typically applied at a different spatial scale. The scale selection may have a crucial role as habitat segregation may be discovered on some but not all spatial scales, even within the same polyploid complex (Halverson et al., 2008; Richardson and Hanks, 2011). Even though several environmental factors operating at distinct spatial scales could be affecting cytotype distribution patterns simultaneously (e.g. large-scale effect of climate, small-scale effect of soil quality), ecological comparisons of cytotypes across several spatial scales have only exceptionally been applied (Laport et al., 2013; Kirchheimer et al., 2016).

Thirdly, significant associations between probability of a cytotype occurrence and values of some environmental variable do not always mean a causal link. In heterogeneous environments, spatial segregation of cytotypes at within-population level can be easily confounded with their niche differentiation, although the same pattern often results from a limited dispersal capacity and/or vegetative propagation (Baack, 2005a; Kao, 2007). When this is the case, inconsistent or contrasting associations between environmental variables and a local frequency of cytotype occurrence may be detected across several sites (Kao and Parker, 2010). The relevance of habitat segregation for cytotype coexistence should be tested either directly *in situ*, using reciprocal transplants of cytotypes (Baack and Stanton, 2005; Buggs and Pannell, 2007; Martin and Husband, 2013), or through testing the direct effect of selected environmental factors on the fitness of particular cytotypes (Lumaret et al., 1987). However, this was only rarely done.

3.3 Temporal dynamics of cytotype coexistence

Frequent incidence of mixed-ploidy populations, documented in many ploidy-heterogeneous species (e.g., *Galax urceolata*, Burton and Husband, 1999; *Arnica cordifolia*, Kao, 2007; *Allium oleraceum*, Duchoslav et al., 2010; *Senecio carniolicus* s.l., Sonnleitner et al., 2010, *Gymnadenia conopsea* agg., Trávníček, Kubátová, et al., 2011; *Andropogon gerardii*, McAllister et al., 2015), rises questions on the fate of local ploidy mixtures and even spatio-temporal dynamics of the entire contact zones. When focusing on the time scale of decades (in order to avoid dramatic changes of large-scale environmental conditions), we could expect either more-or-less stable coexistence of cytotypes or, alternatively, a dynamic landscape-level mosaic where the extirpation of transient mixed-ploidy populations is balanced by formation of new ploidy mixtures (through recurrent neopolyploid formation and/or immigration of cytotypes from other populations). The only way of disentangling these two alternatives is provided by repeated observations of temporal development of ploidy mixtures (Buggs, 2007).

From a handful of available studies on temporal dynamics of ploidy coexistence, the most comprehensive evidence comes from two diploid – hexaploid contact zones of *Mercurialis annua* in Spain, where diploids are consistently displacing hexaploids as a result of asymmetrical reproductive interference and pollen swamping associated with differences in their sexual systems (Buggs and Pannell, 2006). This frontal shift in ploidy composition moved forward by 80 and 200 km in the two zones, respectively, over a period of four decades (Buggs and Pannell, 2006). A shift in ploidy composition of Central-European populations of *Centaurea stoebe*, reflecting human-mediated spread of tetraploids into previously diploid-only areas, resulted either in local displacement of diploids by tetraploids (Welss et al., 2008) or, alternatively, in their more-or-less stable coexistence in a mosaic of diploid-inhabited and tetraploid-inhabited sites that differed considerably in the degree of anthropogenic influence (Mráz et al., 2012; Otisková et al., 2014). A shift in regional distribution of cytotypes of *Vicia cracca*, where rare diploid populations completely vanished from the tetraploid-dominated areas over four decades, remained unexplained (Trávníček et al., 2010). On the other hand, the distribution and size of hexaploid and enneaploid tussocks of *Andropogon gerardii* within mixed-ploidy populations in Colorado has not changed much after four years (Keeler, 2004). Although a contrasting efficiency of generative reproduction between the cytotypes would strongly suggest the hexaploid superiority, sympatric cytotype coexistence is facilitated by a long life-span of the plant (Keeler and Davis, 1999; Keeler, 2004).

While it is difficult to make any generalizations yet, the cytotype coexistence may, at least in some cases, exhibit substantial temporal dynamics. However, even if the cytotype coexistence within populations was fundamentally unstable and led inevitably to fixation of one of the cytotypes, this process could be either delayed (e.g. due to plant perennation and longevity) or compensated by the origin of new ploidy mixtures, allowing sympatric cytotype occurrence at least within shorter time frames. That brings up an interesting question. Do inter-cytotype interactions in mixed-ploidy populations and/or contact zones last long enough to leave significant traces in the evolutionary history of participating cytotypes?

Mating between the coexisting cytotypes may lead to inter-ploidy gene flow and influence the level of genetic diversity, especially in secondary contact zones of differentiated cytotypes. Inter-ploidy gene flow was reported from the populations of e.g. *Senecio carniolicus* s.l. (Hülber et al., 2015), *Arabidopsis arenosa* (Arnold et al., 2015), *Dactylorhiza* spp. (Aagaard et al., 2005; Ståhlberg, 2009; De hert et al., 2012), and *Coffea* spp. (Mahé et al., 2007). The genetic exchange between cytotypes may ultimately lead to a transfer of adaptive traits, as was demonstrated in *Senecio* (Chapman and Abbott, 2010). Alternatively, given that inter-ploidy mating typically has a negative effect on the fitness of cytotypes (Ramsey and Schemske, 1998), selection may act upon the cytotypes co-occurring in contact zones and, as a way of increasing pre-zygotic reproductive isolation, result in more pronounced differences in their biological traits than observed in allopatry (i.e. the process of reinforcement; Van Dijk and Bijlsma, 1994; Nuismer and Cunningham, 2005). Higher selfing rates were observed in parapatric 2x – 4x populations of *Arrhenatherum elatius* compared to allopatric populations of the respective cytotypes (Petit et al., 1997). Similarly, selection likely favoured divergence in flowering time between co-occurring diploids and tetraploids of *Heuchera grossulariifolia* (Nuismer and Cunningham, 2005) and *Plantago media* (Van Dijk and Bijlsma, 1994). A shift in ecological niches of the co-occurring cytotypes may lead to the lower degree of their overlap in sympatric or parapatric than in allopatric populations, and such niche displacement was documented in diploid – polyploid contact zones of *Anthoxanthum alpinum* (Felber-Girard et al., 1996), *Dactylorhiza maculata* s.l. (Ståhlberg, 2009), and *Senecio carniolicus* s.l. (Sonnleitner et al., 2016).

4. Objectives and model plant systems

The main aim of the thesis is to improve our understanding of mechanisms promoting ploidy coexistence in natural populations by addressing several understudied aspects that have been thoroughly introduced in the previous chapter. To make progress, careful selection of the appropriate model plant systems with specific, and often unique features is necessary. I chose two mixed-ploidy species with predominating diploid and tetraploid cytotypes: the serpentine endemic *Knautia serpentinicola* (encompassing a rare case of a primary contact of recently derived cytotypes) and the common weed *Tripleurospermum inodorum* (one of few documented examples of a ploidy heterogeneous annual), both are introduced in the following text. The unique aspects of the selected model species may provide innovative evidence but, on the other hand, make any generalizations of the assessed patterns difficult. In order to cope with that, the new findings have been critically compared with the existing work in a review of the ecological and evolutionary aspects of ploidy coexistence that constitutes the final, fifth paper of this thesis.

General objectives of the thesis are as follows:

- What can we learn from a primary contact zone of closely related diploids and polyploids about the phenotypic effects of genome doubling and the conditions allowing successful establishment of polyploid mutants?
- How important is the role of environmental heterogeneity for facilitating cytotype coexistence and does it change with varying spatial scale under survey?
- Is it possible to capture temporal dynamics of ploidy coexistence within reasonable time frames in natural populations of an annual mixed-ploidy species?
- Based on a survey of the available literature, do cytotype distribution patterns and mechanisms of ploidy coexistence follow some general trends across mixed-ploidy species? What are the gaps in our knowledge that should be further addressed?

4.1 Model species Knautia serpentinicola

Knautia serpentinicola Smejkal ex Kolář, Z. Kaplan, J. Suda et Štech is a recently described member of the field scabious group (*Knautia arvensis* agg., Caprifoliaceae). The species is endemic to Central Europe; only four spatially isolated populations in Bohemia and northern Bavaria are known to date (Kaplan, 1998; Kolář et al., 2015). It inhabits open pine forests on serpentine outcrops, i.e. relict habitats with low levels of interspecific competition that host species assemblages persisting from the early Holocene (Štěpánek, 1997). *Knautia serpentinicola* occurs exclusively at serpentine soils, substrates well-known to impose numerous challenges to plant life. Due to their low calcium to magnesium ratio, deficiency of essential macronutrients, increased (even toxic) concentrations of heavy metals, and low water-holding capacity, serpentine soils can be colonized only by (pre-)adapted biota (Proctor and Woodell, 1975; Brady et al., 2005; Kazakou et al., 2008).

Of the four existing localities, three are occupied by exclusively diploid ($2n = 2x = 20$) populations but one, in the Slavkovský les Mts. (W Bohemia, Czech Republic), is mixed-ploidy and aside from diploids harbours also individuals of an autotetraploid cytotype ($2x = 4x = 40$; Kolář et al., 2009). The two cytotypes are morphologically (Kaplan, 1998; Kolář et al., 2015) and genetically (Kolář et al., 2012) indistinguishable from each other, which is in line with the local and presumably recent (postglacial) origin of tetraploids from the coexisting diploid race. These circumstances make the mixed-ploidy population of *K. serpentinicola* in the Slavkovský les Mts. one of the few well-documented cases of a primary contact zone (*sensu* Petit et al., 1999).

The species is a rhizomatous perennial herb with a well-developed basal leaf rosette and sparsely branched leafy stems of height up to 60 cm. Pink to deeply purple tetramerous flowers are clustered in terminal flower heads and are pollinated by insect (Kolář et al., 2015). Reproductive biology of the species is not yet well known. Even though proterandry and gynodiecy tend to promote outcrossing in most members of the genus (Ehrendorfer, 1962), self-compatibility was confirmed experimentally in *Knautia arvensis* agg. (Vange, 2002). The plants spread either by ant-distributed one-seeded achenes or through vigorous clonal growth based on rhizome elongation and formation of accessory leaf rosettes (Štěpánek, 1997). Previous work has shown that both cytotypes of *K. serpentinicola* are well adapted to harsh conditions of serpentine sites by their physiology (Kolář et al., 2014) and symbiosis with arbuscular mycorrhizal fungi (Doubková et al., 2012).

Specific questions:

(1) What is the spatial structure of cytotypes in the primary contact zone and in fine-scale mixed-ploidy patches? Does it contribute to facilitating cytotype coexistence?

(2) Is the diploid – tetraploid coexistence governed by different habitat preferences of the two cytotypes? If so, does environmental heterogeneity shape cytotype coexistence even within fine-scale mixed-ploidy patches?

(3) Are there any differences in fitness-related traits between the two cytotypes in cultivation, likely reflecting the effect of genome doubling?

(4) Do experimentally cultivated diploids and tetraploids differ in their response to main environmental factors affecting plant growth at their native site (i.e. presence of inhospitable substrate and intensity of interspecific competition)?

4.2 Model species **Tripleurospermum inodorum**

Scentless mayweed [*Tripleurospermum inodorum* (L.) Sch. Bip., syn. *Matricaria perforata* Mérat, Asteraceae] is a common and widely distributed weed of arable land and other man-disturbed sites. Its native range covers most of Europe and Western Asia and it has been introduced to North America and some other temperate regions (Kay, 1976, 1994; Woo et al., 1991). The species has a wide ecological amplitude and, interestingly, even in its native range occupies almost exclusively secondary man-made habitats (Kay, 1994).

Two morphologically indistinguishable cytotypes, diploid ($2n = 2x = 18$) and tetraploid ($2n = 4x = 36$), have been reported from both the native and the introduced ranges (Kay, 1969; Woo et al., 1991). Diploids predominate in western (i.e., oceanic) parts of Europe whereas tetraploids prevail in more continental, eastern parts of the continent (Kay, 1969). A diffuse contact zone of both cytotypes extends from north-eastern France through Germany to Poland, but no mixed-ploidy populations have been reported (Rottgardt, 1956; Kay, 1969; Lankosz-Mróz, 1976). Interploidy breeding barriers seem to be weak, as artificial crosses of di- and tetraploids resulted in triploid seeds (Kay, 1965). An autopolyploid origin has been suggested for the tetraploid cytotype (Arora and Madhusoodanan, 1981).

Tripleurospermum inodorum is an annual herb with finely dissected tripinnate leaves and 0.2–0.7 m tall flowering stems terminated by one to a few hundred capitula (Kubát, 2004). Plants are phenotypically plastic and can vary extremely in their size and habit. The species is insect-pollinated and usually self-incompatible (Kay, 1965; Woo et al., 1991); however, self-pollination in some populations has been reported (Kay, 1969). A typical plant produces thousands of achenes that lack any obvious adaptations for long-distance dispersal but can get readily dispersed together with mud or soil adhering to the wheels of farm vehicles or the hooves of farm animals. Sexual reproduction is the only means of propagation, as the scentless mayweed is incapable of vegetative reproduction (Kay, 1994). Its achenes can survive for at least ten years buried in soil (Kay, 1994), which suggests that it develops a permanent soil seed bank (Bowes et al., 1995). Germination and the formation of leaf rosettes takes place both in autumn and spring, resulting in a winter annual, spring annual or, less frequently, short-lived perennial life cycle (Woo et al., 1991; Kubát, 2004).

Specific questions:

(1) How common is ploidy coexistence in this annual weed? Is there any consistent trend in the temporal development of local ploidy mixtures?

(2) Given the differences in monoploid genome size between di- and tetraploids (inferred during a pilot study), what is the frequency of spontaneous polyploid mutants in natural populations?

(3) Is cytotype diversity at the seedling stage comparable with that in adult plants?

(4) Is the distribution of *T. inodorum* diploids and tetraploids in Europe driven by climatic conditions? If so, where can we predict uniform-ploidy areas and where co-occurrence of the two cytotypes?

(5) Do diploids and tetraploids within the contact zone differ in their preferences for habitat type and/or local ecological conditions? What environmental setting favors the local cytotype coexistence?

5. Methods

Several methods were applied to achieve the objectives of the study. The key technique was DAPI flow cytometry, a fast and cost-effective method enabling relative genome size measurements and ploidy identification of thousands of samples. Interpretation of the flow-cytometric data was always based on karyologically studied individuals. Depending on the study aims, ploidy screening was applied at different spatial scales (from within-population to continental), repeated at the same site to capture local temporal dynamics, and/or employed on different ontogenetic stages of a model plant (e.g. seedlings vs. adults). Various complementary approaches were used for comparing the habitat requirements of different cytotypes. Local environmental conditions, described by ecological indicator values derived from the species composition of vegetation in either uniform-ploidy or mixed-ploidy populations, were used for ecological niche modelling. At larger spatial scales, georeferenced occurrences of particular cytotypes enabled extracting climate data from the Worldclim database. Climatic variables were used for niche comparisons and for predictions of cytotype distribution patterns using maximum entropy modelling. Finally, the field-collected data were supplemented with experimental greenhouse cultivation of cytotypes in different substrate types and under contrasting levels of interspecific competition. The effect of each environment-reflecting treatment could be tested either separately or in combination with other factors, which was convenient for the identification of possible environmental drivers of cytotype coexistence.

6. Key results and conclusions

Particular papers included in the thesis are referred to in the following text by their corresponding Roman numerals (e.g., P–I = Paper I).

The presented thesis aims mainly at getting novel insights into the understudied aspects of polyploid speciation. Following the premise that employing new, convenient methods and/or studying plant model systems with unique features might help to address some gaps in the current knowledge, I focus at several, *a priori* selected topics. These topics are distributed across all the main "stages" of polyploid evolution and encompass the rate of spontaneous polyploidization in nature, establishment of polyploid mutants in diploid parental populations, drivers of cytotype coexistence in contact zones, and, finally, the evolutionary consequences of cytotype coexistence in secondary contact zones.

6.1 Polyploid origin

In our review of cytotype diversity in natural populations of 69 mixed-ploidy species, rare cytotypes among seeds or adults were occasionally reported from otherwise uniform-ploidy populations, evidencing the *in situ* formation of new polyploids (**P–V**). The *Knautia serpentinicola* populations in a primary contact zone of diploid and tetraploid cytotypes were no exception as two minority-cytotype individuals (3x and 6x) were discovered among 4,399 plants, accounting for only 0.05% of sampled individuals. The triploid and hexaploid individuals most likely originated via syngamy of reduced and unreduced gametes in diploid and tetraploid sub-populations, respectively (**P–I**). However, the pathways of minority cytotype origin cannot always be reliably assessed, especially if inter-ploidy hybridization and/or cytotype immigration could lead to the same pattern as local spontaneous polyploidization. A unique opportunity was provided by the profound 19% difference in monoploid genome size of *Tripleurospermum inodorum* diploids and tetraploids, enabling the easy distinction of neopolyploid mutants from long-established polyploids during routine flow-cytometric ploidy screening. Neopolyploid mutants (4x, 6x) were extremely rare among the 11,018 *T. inodorum* individuals sampled from natural populations (approximately 0.04%; **P–III**). Interestingly, the rates of spontaneous polyploidization are very similar in the two studied model taxa, and are an order of magnitude higher than those predicted for autopolyploids by Ramsey and Schemske (1998).

6.2 Polyploid establishment

In *T. inodorum*, the neopolyploids always occurred solitarily, without any signs of their further spread, and were probably restricted to first generation (**P–III**). Several factors including mostly annual life cycle, the virtual lack of clonal propagation, and occasional self-incompatibility suggest that such neopolyploids are facing a strong minority disadvantage (Levin, 1975; Husband, 2000), which may efficiently prevent their local establishment. Therefore, the conditions that initially led to the origin of now well-established *T. inodorum* tetraploids might have been very rare and/or specific. In spite of the substantial effort attributed to unveiling the mechanisms of successful neopolyploid establishment in various mixed-ploidy species, our current knowledge is mostly constrained by studying the outputs of this process, i.e., by comparing long-established polyploids with their diploid relatives (**P–V**). Since the direct effects of genome doubling may be in long-established polyploids confounded with other changes gradually accumulating after the polyploidization event, the initial fitness advantage of new polyploids and/or their ecological divergence from the diploid progenitors might be overestimated (Ramsey, 2011). A new insight into the topic was provided by studying diploids and their sympatric, recently derived autotetraploids in the primary contact zone of *K. serpentinicola* cytotypes in the Slavkovský les Mts. (**P–I**, **P–II**). A comparison of habitat preferences of the co-occurring cytotypes have failed to detect any signs of ecological sorting of cytotypes at the 2x – 4x contact. Instead, the ploidy coexistence seemed to be facilitated by prominent spatial segregation of diploids and tetraploids resulting from non-adaptive processes (i.e. the founder effect, limited dispersal ability, and intense clonal growth; **P–I**). Ploidy-screening data have shown that apart from their successful establishment, *K. serpentinicola* tetraploids even managed to considerably outnumber their diploid progenitors and expanded within the serpentine area, from sparse and long-term stable forests (where they only co-occur with diploids) towards newer forest plantations (**P–I**). The possible drivers of the tetraploid success were discovered during *ex situ* experimental cultivations: the tetraploid individuals attained significantly higher values of both aboveground and belowground biomass compared to their diploid progenitors (**P–II**). The higher aboveground-biomass values could be attributed to greater leaf size in tetraploids, a difference that emerged early in the *Knautia* ontogeny and was likely a consequence of the so-called "gigas effect" (an increase in polyploid cell size that translates into increased organ size; Stebbins, 1971). Moreover, our results showed that the two cytotypes differ in their phenotypic response to key environmental factors. Provided enough nutrients, tetraploids can be more successful in tolerating interspecific competition than their diploid progenitors (**P–II**). Interestingly, this helps explaining the observed successful tetraploid establishment at the natural site. Their superior performance might have allowed the tetraploids to colonize human-affected and more nutrient-rich forest plantations, providing them an escape from interactions with their diploid progenitors.

6.3 Mechanisms of ploidy coexistence

Many plant species retain cytotype diversity, at least in some parts of their distributional range. In our literature survey we revealed that this diversity is relatively high as the 69 species investigated in sufficient detail comprised on average 3.5 cytotypes (range = 2 to 8). Local cytotype coexistence was documented in most mixed-ploidy species, occurring on average in 16.1% of sampled populations (**P–V**). Even though recurrent polyploid origins may contribute to cytotype diversity within populations, the majority of ploidy mixtures resulted from a secondary contact of already established "dominant" cytotypes, e.g. as a consequence of past species-range dynamics (**P–V**). The frequency-dependent mating disadvantage, that efficiently prevents local establishment of polyploid mutants, should also act on cytotypes coexisting in contact zones and lead to gradual fixation of the locally more common cytotype (Levin, 1975). Numerous mechanisms facilitating local cytotype coexistence have been suggested (see Chapter 2.2), however, only a few of these were sufficiently explored in most surveyed mixed-ploidy species (**P–V**). The most frequently reported was spatial segregation of intraspecific cytotypes on various spatial scales, which usually stemmed from niche differentiation of cytotypes, their colonization history, and/or limited dispersal. It should be noted that spatial segregation have, to a certain degree, contributed to cytotype coexistence in both model taxa of this thesis (**P–I, P–III**). Our literature survey also pointed out the importance of asexual reproduction for ploidy coexistence. Not only was the incidence of mixed-ploidy populations twice as common in species with predominantly asexual reproduction (26.7% vs. 14.6%) but such species could harbor relatively high frequencies of odd-ploidy cytotypes (**P–V**).

6.4 Niche differentiation of cytotypes

Niche differentiation of coexisting cytotypes was considered important by authors in approximately one third of the surveyed species; however, only exceptionally were these conclusions supported by experimental comparisons of plant fitness in contrasting environments (**P–V**). Due to non-random distribution of cytotypes, environmental variables may be correlated with the probability of a cytotype occurrence even when there is no causal link. In order to cope with this pitfall, we have 1) experimentally compared the performance of *Knautia* cytotypes under contrasting levels of two most important environmental factors affecting plant growth at the locality of their origin (**P–II**), and 2) looked for the constancy of habitat preferences of *Knautia* and *Tripleurospermum* cytotypes both across different spatial scales and among replicate settings within a particular spatial scale (**P–I**, **P–IV**). While *K. serpentinicola* diploids and tetraploids exhibited the same habitat preferences when uniform-ploidy patches (sub-populations) at or near the ploidy-contact boundary were compared, significant inter-ploidy differences in associated floristic composition were observed at the finest spatial scale, within two mixed-ploidy patches (**P–I**). However, none of the accompanying plant species that were significantly associated with either di- or tetraploids at the finest scale showed a consistent pattern between the two mixed-ploidy patches. This suggests that seeming habitat differentiation was likely an artefact of substantial spatial clustering of cytotypes in mixed-ploidy patches. On the other hand, cultivation of both cytotypes under contrasting environmental settings have revealed that tetraploids are better adapted to nutrient-rich habitats. Such conditions are provided *in situ* by newer forest plantations that are indeed occupied exclusively by tetraploids, while both cytotypes meet only in adjacent long-term stable forests (**P–II**, see above). In *T. inodorum*, we investigated environmental factors driving the occurrence of diploid and tetraploid cytotypes in Europe at several spatial scales, ranging from population to continental level. Large-scale analyses have revealed that diploids prefer areas with oceanic climate, whereas tetraploids favor areas with the contrasting continental climate (**P–IV**), which is in line with the occasionally reported association between polyploidy and increased tolerance to arid environments (Treier et al., 2009; Manzaneda et al., 2012; Hao et al., 2013). Climate likely acts as an important driver of cytotype distribution patterns at larger spatial scales (Europe, Central Europe), delimiting the position and breath of the contact zone. Within the area where climatic conditions allow co-occurrence of diploids and tetraploids, i.e. in the contact zone, we did not observe any constant differences in ecological preferences of the co-existing cytotypes, in spite of our thorough search conducted independently in three regions (**P–IV**). It is thus likely, at regional and finer scales, that non-random migration patterns caused by human activities and the founder effect are more important determinants of cytotype distribution patterns than environmental heterogeneity. Such non-adaptive explanations for cytotype coexistence have received increased attention in the recent years (**P–V**). Also, no ecological factors aside from the general habitat suitability seemed to constrain the formation of local ploidy mixtures, which corresponds with the observed ubiquity of mixed-ploidy populations in *T. inodorum* (**P–III**). Collectively, our studies (**P–I**, **P–IV**) have demonstrated that independent replicates and/or of across-scale comparisons may significantly improve the performance of ecological niche assessments, which is especially convenient in cases when targeted experimental study is problematic (e.g. the European-scale climatic differentiation of *T. inodorum* cytotypes; **P–IV**). Moreover, different environmental forces and biological processes may shape cytotype distribution patterns at different spatial scales.

6.5 Dynamics of ploidy coexistence

A relatively high incidence of mixed-ploidy populations in ploidy heterogeneous species (see above) raises the question of whether local ploidy mixtures are stable over the long term or in a transient state of coexistence. However, our knowledge of the temporal development of local ploidy mixtures is very limited and likely constrained by the prevalence of (long-lived) perennials among mixed-ploidy species (**P–V**). In order to get new insight into the topic, we initiated our investigation of the ploidy heterogeneous annual weed *Tripleurospermum inodorum*. Repeated ploidy screening in mixed-ploidy populations of this species have documented considerable between-year oscillations in cytotype frequencies (and in the overall population abundance) and thus a dynamic nature of cytotype coexistence (**P–III**). To our knowledge, this was the first attempt to document temporal changes in cytotype composition *in situ*, at the within-population scale. On the other hand, both diploids and tetraploids usually persisted locally up to the last census (after 1-5 years). The surprising persistence of mixed-ploidy populations in an annual species was likely driven by a combined effect of permanent soil seed banks, repeated immigration of seeds of particular cytotypes from surrounding populations, and recurring local disturbances. Even though permanent soil seed banks are often neglected in polyploid studies (but see Hahn et al., 2012), our data suggest they may play an important role in stabilizing mixed-ploidy populations, at least in short-lived plants. Interestingly, the dynamic nature of cytotype coexistence was also apparent at a regional scale, as the striking shortage of uniformly diploid populations (compared to mixed-ploidy and uniformly tetraploid populations) suggested ongoing spread of tetraploids within all three studied areas (**P–III**), which might potentially lead to shift in spatial position of the contact zone. Certain, albeit limited, evidence of the dynamics of ploidy coexistence was also documented in the long-lived perennial *Knautia serpentinicola*. In three out of four mixed-ploidy patches, coexisting di- and tetraploids differed in proportions of the main life-stage categories (juvenile, vegetative and flowering plants), which may indicate that one of the coexisting cytotypes is either a recent immigrant or gradually in decline (**P–I**).

6.6 Evolutionary consequences of ploidy coexistence

Finally, considering the ploidy coexistence only a temporal situation, do inter-cytotype interactions in mixed-ploidy populations and/or contact zones last long enough to leave significant traces in the evolutionary history of participating cytotypes? A high potential for inter-ploidy gene flow was expected in the diploid – tetraploid contact zone of *T. inodorum* due to its mosaic-like spatial structure, common incidence of mixed-ploidy populations (up to 43% in one of the study regions), and a relatively high frequency of triploid hybrids (mean rate = 8%), which are partially fertile (**P–III**). Theoretically, the triploids may act as mediators of bi-directional gene flow across ploidy barrier (Husband, 2004). A non-direct evidence of the inter-ploidy gene flow was provided by ploidy analysis of offspring from such mixed-ploidy sites. The offspring exhibited striking variation in DNA content, which could be attributed to ongoing reproductive interactions between triploid hybrids and their parental cytotypes (**P–III**). Inter-ploidy gene flow has been recently corroborated by pilot results of our molecular analyses (data not presented). Given the likely high genetic differentiation of *T. inodorum* cytotypes (inferred from substantial differences in monoploid genome sizes and the recent molecular data), the inter-ploidy gene flow might be a source of genetic novelty and thus even confer an adaptive advantage (e.g. through a transfer of adaptations). On the other hand, inter-ploidy mating typically has an immediate negative effect on the fitness of cytotypes, which may lead to selection for minimizing the reproductive interactions of cytotypes in contact zones (i.e., character displacement, reinforcement; **P–V**). In *T. inodorum*, we looked for signs of niche displacement, i.e. a shift in ecological niches of cytotypes leading to lower degree of their overlap in sympatric than in allopatric populations, which was repeatedly documented in diploid – polyploid contact zones (Felber-Girard et al., 1996; Ståhlberg, 2009; Sonnleitner et al., 2016). However, the range of ecological conditions enabling the coexistence of *T. inodorum* diploids and tetraploids was not reduced in regions of sympatric cytotype occurrence; contrary to that, ploidy mixtures were found to occupy almost the entire spectrum of ecological niches suitable for the species (**P–IV**). Even though niche displacement was not observed in *T. inodorum*, other adaptive changes limiting the inter-ploidy mating in sympatry cannot yet be ruled out without targeted study.

7. References

AAGAARD, S.M.D., S.M. SÅSTAD, J. GREILHUBER, and A. MOEN. 2005. A secondary hybrid zone between diploid *Dactylorhiza incarnata* ssp. *cruenta* and allotetraploid *D. lapponica* (Orchidaceae). *Heredity* 94: 488–496.

ARNOLD, B., S.-T. KIM, and K. BOMBLIES. 2015. Single geographic origin of a widespread autotetraploid *Arabidopsis arenosa* lineage followed by interploidy admixture. *Molecular Biology and Evolution* 32: 1382–1395.

ARORA, O.P., and K.J. MADHUSOODANAN. 1981. Nature of tetraploidy in *Matricaria inodora* L. *Cytologia* 46: 773–779.

ARVANITIS, L., C. WIKLUND, and J. EHRLÉN. 2008. Plant ploidy level infuences selection by butterfly seed predators. *Oikos* 117: 1020–1025.

BAACK, E.J. 2004. Cytotype segregation on regional and microgeographic scales in snow buttercups (*Ranunculus adoneus*: Ranunculaceae). *American Journal of Botany* 91: 1783–1788.

BAACK, E.J. 2005a. Ecological factors influencing tetraploid establishment in snow buttercups (*Ranunculus adoneus*, Ranunculaceae): Minority cytotype exclusion and barriers to triploid formation. *American Journal of Botany* 92: 1827–1835.

BAACK, E.J. 2005b. To succeed globally, disperse locally: Effects of local pollen and seed dispersal on tetraploid establishment. *Heredity* 94: 538–546.

BAACK, E.J., and M.L. STANTON. 2005. Ecological factors influencing tetraploid speciation in snow buttercups (*Ranunculus adoneus*): Niche differentiation and tetraploid establishment. *Evolution* 59: 1936–1944.

BARKER, M.S., N. ARRIGO, A.E. BANIAGA, Z. LI, and D.A. LEVIN. 2016. On the relative abundance of autopolyploids and allopolyploids. *New Phytologist* 210: 391–398.

BARKER, M.S., B.C. HUSBAND, and J. CHRIS PIRES. 2016. Spreading winge and flying high: The evolutionary importance of polyploidy after a century of study. *American Journal of Botany* 103: 1139–1145.

BARRINGER, B.C. 2007. Polyploidy and self-fertilization in flowering plants. *American Journal of Botany* 94: 1527–1533.

BICKNELL, R.A., and A.M. KOLTUNOW. 2004. Understanding apomixis: Recent advances and remaining conundrums. *The Plant Cell* 16: 228–245.

BOWES, G.G., A.G. THOMAS, and L.P. LEFKOVITCH. 1995. Changes with time in the germination of buried scentless chamomile (*Matricaria perforata* Mérat) seeds. *Canadian Journal of Plant Science* 75: 277–281.

BRADY, K.U., A.R. KRUCKEBERG, and H.D. BRADSHAW. 2005. Evolutionary ecology of plant adaptation to serpentine soils. *Annual Review of Ecology, Evolution, and Systematics* 36: 243–266.

BRETAGNOLLE, F., and R. LUMARET. 1995. Bilateral polyploidization in *Dactylis glomerata* L. subsp. *lusitanica*: Occurrence, morphological and genetic characteristics of first polyploids. *Euphytica* 84: 197–207.

BRETAGNOLLE, F., and J.D. THOMPSON. 2001. Phenotypic plasticity in sympatric diploid and autotetraploid *Dactylis glomerata*. *International Journal of Plant Sciences* 162: 309–316.

BROCHMANN, C., A.K. BRYSTING, I.G. ALSOS, L. BORGEN, H.H. GRUNDT, A.C. SCHEEN, and R. ELVEN. 2004. Polyploidy in arctic plants. *Biological Journal of the Linnean Society* 82: 521–536.

BUGGS, R.J.A. 2007. Empirical study of hybrid zone movement. *Heredity* 99: 301–312.

BUGGS, R.J.A., and J.R. PANNELL. 2007. Ecological differentiation and diploid superiority across a moving ploidy contact zone. *Evolution* 61: 125–140.

BUGGS, R.J.A., and J.R. PANNELL. 2006. Rapid displacement of a monoecious plant lineage is due to pollen swamping by a dioecious relative. *Current Biology* 16: 996–1000.

BURTON, T.L., and B.C. HUSBAND. 1999. Population cytotype structure in the polyploid *Galax urceolata* (Diapensiaceae). *Heredity* 82: 381–390.

CASTRO, S., J. LOUREIRO, T. PROCHÁZKA, and Z. MÜNZBERGOVÁ. 2012. Cytotype distribution at a diploid-hexaploid contact zone in *Aster amellus* (Asteraceae). *Annals of Botany* 110: 1047–1055.

COLLINS, A.R., R. NADERI, and H. MUELLER-SCHAERER. 2011. Competition between cytotypes changes across a longitudinal gradient in *Centaurea stoebe* (Asteraceae). *American Journal of Botany* 98: 1935–1942.

COYNE, J.A., and H.A. ORR. 2004. Speciation. Sinauer Associates, Sunderland.

VAN DIJK, P., and T. BAKX-SCHOTMAN. 1997. Chloroplast DNA phylogeography and cytotype geography in autopolyploid *Plantago media*. *Molecular Ecology* 6: 345–352.

VAN DIJK, P., and R. BIJLSMA. 1994. Simulations of flowering time displacement between two cytotypes that form inviable hybrids. *Heredity* 72: 522–535.

VAN DIJK, P., M. HARTOG, and W. VAN DELDEN. 1992. Single cytotype areas in autopolyploid *Plantago media* L. *Biological Journal of the Linnean Society* 46: 315–331.

DOLEŽEL, J., J. GREILHUBER, and J. SUDA. 2007. Flow cytometry with plant cells: Analysis of genes, chromosomes and genomes. John Wiley & Sons, Weinheim.

DOUBKOVÁ, P., J. SUDA, and R. SUDOVÁ. 2012. The symbiosis with arbuscular mycorrhizal fungi contributes to plant tolerance to serpentine edaphic stress. *Soil Biology and Biochemistry* 44: 56–64.

DUCHOSLAV, M., L. ŠAFÁŘOVÁ, and F. KRAHULEC. 2010. Complex distribution patterns, ecology and coexistence of ploidy levels of *Allium oleraceum* (Alliaceae) in the Czech Republic. *Annals of Botany* 105: 719–735.

EHRENDORFER, F. 1962. Beiträge zur Phylogenie der Gattung *Knautia* (Dipsacaceae), I. Cytologische Grundlagen und allgemeine Hinweise. *Österreichische Botanische Zeitschrift* Z276–343.

EHRENDORFER, F. 1980. Polyploidy and distribution. *In* W. H. Lewis [ed.], Polyploidy: Biological relevance, 45–60. Plenum Press, New York.

FELBER-GIRARD, M., F. FELBER, and A. BUTTLER. 1996. Habitat differentiation in a narrow hybrid zone between diploid and tetraploid *Anthoxanthum alpinum*. *New Phytologist* 133: 531–540.

FLAGEL, L.E., and J.F. WENDEL. 2009. Gene duplication and evolutionary novelty in plants. *New Phytologist* 183: 557–564.

FOWLER, N.L., and D.A. LEVIN. 1984. Ecological constraints on the establishment of a novel polyploid in competition with its diploid progenitor. *The American Naturalist* 124: 703–711.

GLENNON, K.L., M.E. RITCHIE, and K.A. SEGRAVES. 2014. Evidence for shared broad-scale climatic niches of diploid and polyploid plants. *Ecology Letters* 17: 574–582.

GODSOE, W., M.A. LARSON, K.L. GLENNON, and K.A. SEGRAVES. 2013. Polyploidization in *Heuchera cylindrica* (Saxifragaceae) did not result in a shift in climatic requirements. *American Journal of Botany* 100: 496–508.

GRANT, V. 1981. Plant speciation. 2nd ed. Columbia University Press, New York.

HAGBERG, A., and S. ELLERSTRÖM. 1959. The competition between diploid, tetraploid and aneuploid rye: Theoretical and practical aspects. *Hereditas* 45: 369–416.

HAHN, M.A., Y.M. BUCKLEY, and H. MÜLLER-SCHÄRER. 2012. Increased population growth rate in invasive polyploid *Centaurea stoebe* in a common garden. *Ecology Letters* 15: 947–954.

HALVERSON, K., S.B. HEARD, J.D. NASON, and J.O. STIREMAN. 2008. Origins, distribution, and local co-occurrence of polyploid cytotypes in *Solidago altissima* (Asteraceae). *American Journal of Botany* 95: 50–58.

HAO, G.Y., M.E. LUCERO, S.C. SANDERSON, E.H. ZACHARIAS, and N.M. HOLBROOK. 2013. Polyploidy enhances the occupation of heterogeneous environments through hydraulic related trade-offs in *Atriplex canescens* (Chenopodiaceae). *New Phytologist* 197: 970–978.

HARLAN, J.R., and J.M.J. DE WET. 1975. On Ö. Winge and a prayer: The origins of polyploidy. *Botanical Review* 41: 361–390.

HEGARTY, M., J. COATE, S. SHERMAN-BROYLES, R. ABBOTT, S. HISCOCK, and J. DOYLE. 2013. Lessons from natural and artificial polyploids in higher plants. *Cytogenetic and Genome Research* 140: 204–225.

DE HERT, K., H. JACQUEMYN, S. VAN GLABEKE, I. ROLDÁN-RUIZ, K. VANDEPITTE, L. LEUS, and O. HONNAY. 2012. Reproductive isolation and hybridization in sympatric populations of three *Dactylorhiza* species (Orchidaceae) with different ploidy levels. *Annals of Botany* 109: 709–720.

HROUDOVÁ, Z., and P. ZÁKRAVSKÝ. 1993. Ecology of two cytotypes of *Butomus umbellatus* II: Reproduction, growth and biomass production. *Folia Geobotanica and Phytotaxonomica* 28: 413–424.

HÜLBER, K., M. SONNLEITNER, J. SUDA, J. KREJČÍKOVÁ, P. SCHÖNSWETTER, G.M. SCHNEEWEISS, and M. WINKLER. 2015. Ecological differentiation, lack of hybrids involving diploids, and asymmetric gene flow between polyploids in narrow contact zones of *Senecio carniolicus* (syn. *Jacobaea carniolica*, Asteraceae). *Ecology and Evolution* 5: 1224–1234.

HUSBAND, B.C. 2000. Constraints on polyploid evolution: A test of the minority cytotype exclusion principle. *Proceedings of the Royal Society B-Biological Sciences* 267: 217–223.

HUSBAND, B.C. 2004. The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. *Biological Journal of the Linnean Society* 82: 537–546.

HUSBAND, B.C., B. OZIMEC, S.L. MARTIN, and L. POLLOCK. 2008. Mating consequences of polyploid evolution in flowering plants: Current trends and insights from synthetic polyploids. *International Journal of Plant Sciences* 169: 195–206.

HUSBAND, B.C., and D.W. SCHEMSKE. 1997. The effect of inbreeding in diploid and tetraploid populations of *Epilobium angustifolium* (Onagraceae): Implications for the genetic basis of inbreeding depression. *Evolution* 51: 737–746.

HUSBAND, B.C., D.W. SCHEMSKE, T.L. BURTON, and C. GOODWILLIE. 2002. Pollen competition as a unilateral reproductive barrier between sympatric diploid and tetraploid *Chamerion angustifolium*. *Proceedings of the Royal Society B-Biological Sciences* 269: 2565–2571.

CHAPMAN, M.A., and R.J. ABBOTT. 2010. Introgression of fitness genes across a ploidy barrier. *New Phytologist* 186: 63–71.

JERSÁKOVÁ, J., S. CASTRO, N. SONK, K. MILCHREIT, I. SCHÖDELBAUEROVÁ, T. TOLASCH, and S. DÖTTERL. 2010. Absence of pollinator-mediated premating barriers in mixed-ploidy populations of *Gymnadenia conopsea* s.l. (Orchidaceae). *Evolutionary Ecology* 24: 1199–1218.

KAO, R.H. 2007. Asexuality and the coexistence of cytotypes. *New Phytologist* 175: 764–772.

KAO, R.H., and I.M. PARKER. 2010. Coexisting cytotypes of *Arnica cordifolia*: Morphological differentiation and local-scale distribution. *International Journal of Plant Sciences* 171: 81–89.

KAPLAN, Z. 1998. Relict serpentine populations of *Knautia arvensis* s.l. (Dipsacaceae) in the Czech Republic and an adjacent area of Germany. *Preslia* 70: 21–31.

KAY, Q.O.N. 1976. 60. *Matricaria* L. *In* T.G. Tutin, V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentine, S.M. Walters, and D.A. Webb [eds.], Flora Europaea, 4: 165–167. Cambridge University Press, Cambridge.

KAY, Q.O.N. 1965. Experimental and comparative ecological studies of selected weeds. PhD. Thesis, University of Oxford, UK.

KAY, Q.O.N. 1969. The origin and distribution of diploid and tetraploid *Tripleurospermum inodorum* (L.) Schultz Bip. *Watsonia* 7: 130–141.

KAY, Q.O.N. 1994. *Tripleurospermum inodorum* (L.) Schultz Bip. *Journal of Ecology* 82: 681–697.

KAZAKOU, E., P.G. DIMITRAKOPOULOS, A.J.M. BAKER, R.D. REEVES, and A.Y. TROUMBIS. 2008. Hypotheses, mechanisms and trade-offs of tolerance and adaptation to serpentine soils: From species to ecosystem level. *Biological Reviews* 83: 495–508.

KEELER, K.H. 2004. Impact of intraspecific polyploidy in *Andropogon gerardii* (Poaceae) populations. *The American Midland Naturalist* 152: 63–74.

KEELER, K.H., and G.A. DAVIS. 1999. Comparison of common cytotypes of Andropogon gerardii (Andropogoneae, Poaceae). *American Journal of Botany* 86: 974–979.

KENNEDY, B.F., H.A. SABARA, D. HAYDON, and B.C. HUSBAND. 2006. Pollinator-mediated assortative mating in mixed ploidy populations of *Chamerion angustifolium* (Onagraceae). *Oecologia* 150: 398–408.

KIRCHHEIMER, B., C.C.F. SCHINKEL, A.S. DELLINGER, S. KLATT, D. MOSER, M. WINKLER, J. LENOIR, ET AL. 2016. A matter of scale: Apparent niche differentiation of diploid and tetraploid plants may depend on extent and grain of analysis. *Journal of Biogeography* 43: 716–726.

KLIBER, A., and C.G. ECKERT. 2005. Interaction between founder effect and selection during biological invasion in an aquatic plant. *Evolution* 59: 1900–1913.

KOLÁŘ, F., M. DORTOVÁ, J. LEPŠ, M. POUZAR, A. KREJČOVÁ, and M. ŠTECH. 2014. Serpentine ecotypic differentiation in a polyploid plant complex: Shared tolerance to Mg and Ni stress among di- and tetraploid serpentine populations of *Knautia arvensis* (Dipsacaceae). *Plant Soil* 374: 435–447.

KOLÁŘ, F., T. FÉR, M. ŠTECH, P. TRÁVNÍČEK, E. DUŠKOVÁ, P. SCHÖNSWETTER, and J. SUDA. 2012. Bringing together evolution on serpentine and polyploidy: Spatiotemporal history of the diploid-tetraploid complex of *Knautia arvensis* (Dipsacaceae). *PLoS ONE* 7: e39988.

KOLÁŘ, F., Z. KAPLAN, J. SUDA, and M. ŠTECH. 2015. Populations of *Knautia* in ecologically distinct refugia on the Hercynian massif belong to two endemic species. *Preslia* 87: 363–386.

KOLÁŘ, F., M. LUČANOVÁ, P. VÍT, T. URFUS, J. CHRTEK, T. FÉR, F. EHRENDORFER, and J. SUDA. 2013. Diversity and endemism in deglaciated areas: Ploidy, relative genome size and niche differentiation in the *Galium pusillum* complex (Rubiaceae) in Northern and Central Europe. *Annals of Botany* 111: 1095–1108.

KOLÁŘ, F., M. ŠTECH, P. TRÁVNÍČEK, J. RAUCHOVÁ, T. URFUS, P. VÍT, M. KUBEŠOVÁ, and J. SUDA. 2009. Towards resolving the *Knautia arvensis* agg. (Dipsacaceae) puzzle: Primary and secondary contact zones and ploidy segregation at landscape and microgeographic scales. *Annals of Botany* 103: 963–974.

KUBÁT, K. 2004. 33. *Tripleurospermum* Schultz Bip. - heřmánkovec. *In* B. Slavík, and J. Štěpánková [eds.], Květena České republiky, 248–250. Academia, Praha.

LANKOSZ-MRÓZ, M. 1976. Karyological investigations on *Tripleurospermum maritimum* (L.) Koch ssp. *inodorum* (L.) ex Vaarama from Poland. *Acta Biologica Cracoviensia (Series Botanica)* 19: 93–105.

LAPORT, R.G., L. HATEM, R.L. MINCKLEY, and J. RAMSEY. 2013. Ecological niche modeling implicates climatic adaptation , competitive exclusion , and niche conservatism among *Larrea tridentata* cytotypes in North American deserts. *The Journal of the Torrey Botanical Society* 140: 349–363.

LAPORT, R.G., and J. RAMSEY. 2015. Morphometric analysis of the North American creosote bush (*Larrea tridentata*, Zygophyllaceae) and the microspatial distribution of its chromosome races. *Plant Systematics and Evolution* 301: 1581–1599.

LEVIN, D.A. 1975. Minority cytotype exclusion in local plant populations. *Taxon* 24: 35–43.

LEVIN, D.A. 1983. Polyploidy and novelty in flowering plants. *The American Naturalist* 122: 1–25.

LEVIN, D.A. 2002. The role of chromosomal change in plant evolution. Oxford University Press, Oxford.

LEWIS, H. 1967. The taxonomic significance of autopolyploidy. *Taxon* 16: 267–271.

LEWIS, W.H. 1980. Polyploidy in species populations. *In* W. H. Lewis [ed.], Polyploidy: Biological relevance, 103–144. Plenum Press, New York.

LI, B.H., X.M. XU, and M.S. RIDOUT. 2004. Modelling the establishment and spread of autotetraploid plants in a spatially heterogeneous environment. *Journal of Evolutionary Biology* 17: 562–573.

LUMARET, R., J.L. GUILLERM, J. DELAY, A. AIT LHAJ LOUTFI, J. IZCO, and M. JAY. 1987. Polyploidy and habitat differentiation in *Dactylis glomerata* L. from Galicia (Spain). *Oecologia* 73: 436–446.

MACEIRA, N.O., P. JACQUARD, and R. LUMARET. 1993. Competition between diploid and derivative autotetraploid *Dactylis glomerata* L. from Galicia. Implications for the establishment of novel polyploid populations. *New Phytologist* 124: 321–328.

MAHÉ, L., D. LE PIERRÈS, M.-C. COMBES, and P. LASHERMES. 2007. Introgressive hybridization between the allotetraploid *Coffea arabica* and one of its diploid ancestors, *Coffea canephora*, in an exceptional sympatric zone in New Caledonia. *Genome* 50: 316–324.

MANZANEDA, A.J., P.J. REY, J.M. BASTIDA, C. WEISS-LEHMAN, E. RASKIN, and T. MITCHELL-OLDS. 2012. Environmental aridity is associated with cytotype segregation and polyploidy occurrence in *Brachypodium distachyon* (Poaceae). *The New Phytologist* 193: 797–805.

MARTIN, S.L., and B.C. HUSBAND. 2013. Adaptation of diploid and tetraploid *Chamerion angustifolium* to elevation but not local environment. *Evolution* 67: 1780–1791.

MCALLISTER, C., R. BLAINE, P. KRON, B. BENNETT, H. GARRETT, J. KIDSON, B. MATZENBACHER, ET AL. 2015. Environmental correlates of cytotype distribution in *Andropogon gerardii* (Poaceae). *American Journal of Botany* 102: 92–102.

MEIRMANS, P.G., F.G. CALAME, F. BRETAGNOLLE, F. FELBER, and J.C.M. DEN NIJS. 1999. Anthropogenic disturbance and habitat differentiation between sexual diploid and apomictic triploid *Taraxacum* sect. *Ruderalia*. *Folia Geobotanica* 34: 451–469.

MRÁZ, P., S. ŠPANIEL, A. KELLER, G. BOWMANN, A. FARKAS, B. ŠINGLIAROVÁ, R.P. ROHR, ET AL. 2012. Anthropogenic disturbance as a driver of microspatial and microhabitat segregation of cytotypes of *Centaurea stoebe* and cytotype interactions in secondary contact zones. *Annals of Botany* 110: 615–627.

NUISMER, S.L., and B.M. CUNNINGHAM. 2005. Selection for phenotypic divergence between diploid and autotetraploid *Heuchera grossulariifolia*. *Evolution* 59: 1928–1935.

NUISMER, S.L., and J.N. THOMPSON. 2001. Plant polyploidy and non-uniform effects on insect herbivores. *Proceedings of the Royal Society B-Biological Sciences* 268: 1937–1940.

OSWALD, B.P., and S.L. NUISMER. 2011. Neopolyploidy and diversification in *Heuchera grossulariifolia*. *Evolution* 65: 1667–1679.

OTISKOVÁ, V., T. KOUTECKÝ, F. KOLÁŘ, and P. KOUTECKÝ. 2014. Occurrence and habitat preferences of diploid and tetraploid cytotypes of *Centaurea stoebe* in the Czech Republic. *Preslia* 86: 67–80.

PECKERT, T., and J. CHRTEK. 2006. Mating interactions between coexisting diploid, triploid and tetraploid cytotypes of *Hieracium echioides* (Asteraceae). *Folia Geobotanica* 41: 323–334.

PETIT, C., F. BRETAGNOLLE, and F. FELBER. 1999. Evolutionary consequences of diploid-polyploid hybrid zones in wild species. *Trends in Ecology and Evolution* 14: 306–311.

PETIT, C., P. LESBROS, X. GE, and J.D. THOMPSON. 1997. Variation in flowering phenology and selfing rate across a contact zone between diploid and tetraploid *Arrhenatherum elatius* (Poaceae). *Heredity* 79: 31–40.

PROCTOR, J., and S.R.J. WOODELL. 1975. The ecology of serpentine soils. *In* A. MacFadyen [ed.], Advances in ecological research, 255–366. Academic Press, New York.

RAMSEY, J. 2011. Polyploidy and ecological adaptation in wild yarrow. *Proceedings of the National Academy of Sciences of the United States of America* 108: 7096–7101.

RAMSEY, J. 2007. Unreduced gametes and neopolyploids in natural populations of *Achillea borealis* (Asteraceae). *Heredity* 98: 143–150.

RAMSEY, J., and T.S. RAMSEY. 2014. Ecological studies of polyploidy in the 100 years following its discovery. *Philosophical Transactions of the Royal Society B: Biological Sciences* 369: 20130352.

RAMSEY, J., and D.W. SCHEMSKE. 2002. Neopolyploidy in flowering plants. *Annual Review of Ecology and Systematics* 33: 589–639.

RAMSEY, J., and D.W. SCHEMSKE. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* 29: 467–501.

RAUSCH, J.H., and M.T. MORGAN. 2005. The effect of self-fertilization, inbreeding depression, and population size on autopolyploid establishment. *Evolution* 59: 1867–1875.

RICHARDSON, M.L., and L.M. HANKS. 2011. Differences in spatial distribution, morphology, and communities of herbivorous insects among three cytotypes of *Solidago altissima* (Asteraceae). *American Journal of Botany* 98: 1595–1601.

RODRÍGUEZ, D.J. 1996. A model for the establishment of polyploidy in plants. *The American Naturalist* 147: 33–46.

ROTTGARDT, K. 1956. Morphologische, cytologische und physiologische Untersuchungen von Ökotypen in Schleswig-Holstein. *Beiträge zur Biologie der Pflanzen* 32: 225–278.

SEGRAVES, K.A., and J.N. THOMPSON. 1999. Plant polyploidy and pollination: Floral traits and insect visits to diploid and tetraploid *Heuchera grossulariifolia*. *Evolution* 53: 1114–1127.

SEGRAVES, K.A., J.N. THOMPSON, P.S. SOLTIS, and D.E. SOLTIS. 1999. Multiple origins of polyploidy and the geographic structure of *Heuchera grossulariifolia*. *Molecular Ecology* 8: 253–262.

SOLTIS, D.E., V.A. ALBERT, J. LEEBENS-MACK, C.D. BELL, A.H. PATERSON, C. ZHENG, D. SANKOFF, ET AL. 2009. Polyploidy and angiosperm diversification. *American Journal of Botany* 96: 336–348.

SOLTIS, D.E., and P.S. SOLTIS. 1999. Polyploidy: Recurrent formation and genome evolution. *Trends in Ecology and Evolution* 14: 348–352.

SOLTIS, D.E., P.S. SOLTIS, D.W. SCHEMSKE, J.F. HANCOCK, J.N. THOMPSON, B.C. HUSBAND, and W.S. JUDD. 2007. Autopolyploidy in angiosperms : Have we grossly underestimated the number of species? *Taxon* 56: 13–30.

SONNLEITNER, M., R. FLATSCHER, P. ESCOBAR GARCÍA, J. RAUCHOVÁ, J. SUDA, G.M. SCHNEEWEISS, K. HÜLBER, and P. SCHÖNSWETTER. 2010. Distribution and habitat segregation on different spatial scales among diploid, tetraploid and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps. *Annals of Botany* 106: 967–977.

SONNLEITNER, M., K. HÜLBER, R. FLATSCHER, P.E. GARCÍA, M. WINKLER, J. SUDA, P. SCHÖNSWETTER, and G.M. SCHNEEWEISS. 2016. Ecological differentiation of diploid and polyploid cytotypes of *Senecio carniolicus* sensu lato (Asteraceae) is stronger in areas of sympatry. *Annals of Botany* 117: 269–276.

STÅHLBERG, D. 2009. Habitat differentiation, hybridization and gene flow patterns in mixed populations of diploid and autotetraploid *Dactylorhiza maculata* s.l. (Orchidaceae). *Evolutionary Ecology* 23: 295–328.

STEBBINS, G.L. 1971. Chromosomal evolution in higher plants. Edward Arnold, London.

STEBBINS, G.L.J. 1947. Types of polyploids: Their classification and significance. *Advances in Genetics* 1: 403–429.

SUDA, J., and T. HERBEN. 2013. Ploidy frequencies in plants with ploidy heterogeneity: Fitting a general gametic model to empirical population data. *Proceedings of the Royal Society B-Biological Sciences* 280: 20122387.

SUDOVÁ, R., H. PÁNKOVÁ, J. RYDLOVÁ, Z. MÜNZBERGOVÁ, and J. SUDA. 2014. Intraspecific ploidy variation: A hidden, minor player in plant-soil-mycorrhizal fungi interactions. *American Journal of Botany* 101: 26–33.

ŠAFÁŘOVÁ, L., and M. DUCHOSLAV. 2010. Cytotype distribution in mixed populations of polyploid *Allium oleraceum* measured at a microgeographic scale. *Preslia* 82: 107–126.

ŠINGLIAROVÁ, B., J. CHRTEK, I. PLAČKOVÁ, and P. MRÁZ. 2011. Allozyme variation in diploid, polyploid and mixed-ploidy populations of the *Pilosella alpicola* Group (Asteraceae): Relation to morphology, origin of polyploids and breeding system. *Folia Geobotanica* 46: 387–410.

ŠTĚPÁNEK, J. 1997. *Knautia* L. *In* B. Slavík [ed.], Květena České republiky, 6: 543–554. Academia, Praha.

THOMPSON, S.L., and J. WHITTON. 2006. Patterns of recurrent evolution and geographic parthenogenesis within apomictic polyploid Easter daises (*Townsendia hookeri*). *Molecular Ecology* 15: 3389–3400.

TRÁVNÍČEK, P., Z. DOČKALOVÁ, R. ROSENBAUMOVÁ, B. KUBÁTOVÁ, Z. SZELĄG, and J. CHRTEK. 2011. Bridging global and microregional scales: Ploidy distribution in *Pilosella echioides* (Asteraceae) in central Europe. *Annals of Botany* 107: 443–454.

TRÁVNÍČEK, P., A. ELIÁŠOVÁ, and J. SUDA. 2010. The distribution of cytotypes of *Vicia cracca* in Central Europe: The changes that have occurred over the last four decades. *Preslia* 82: 149–163.

TRÁVNÍČEK, P., B. KUBÁTOVÁ, V. ČURN, J. RAUCHOVÁ, E. KRAJNÍKOVÁ, J. JERSÁKOVÁ, and J. SUDA. 2011. Remarkable coexistence of multiple cytotypes of the *Gymnadenia conopsea* aggregate (the fragrant orchid): Evidence from flow cytometry. *Annals of Botany* 107: 77–87.

TREIER, U.A., O. BROENNIMANN, S. NORMAND, A. GUISAN, U. SCHAFFNER, T. STEINGER, and H. MÜLLER-SCHÄRER. 2009. Shift in cytotype frequency and niche space in the invasive plant *Centaurea maculosa*. *Ecology* 90: 1366–1377.

VANGE, V. 2002. Breeding system and inbreeding depression in the clonal plant species *Knautia arvensis* (Dipsacaceae): Implications for survival in abandoned grassland. *Biological Conservation* 108: 59–67.

WEISS-SCHNEEWEISS, H., K. EMADZADE, T.S. JANG, and G.M. SCHNEEWEISS. 2013. Evolutionary consequences, constraints and potential of polyploidy in plants. *Cytogenetic and Genome Research* 140: 137–150.

WELSS, W., P. REGER, and W. NEZADAL. 2008. Zur Verbreitung von *Centaurea stoebe* L. subsp. *stoebe* und *Centaurea stoebe* subsp. *australis* (A. Kern) Greuter (Asteraceae) im Nürnberger Becken. *Regnitz Flora* 2: 44–53.

WENDEL, J.F., S.A. JACKSON, B.C. MEYERS, and R.A. WING. 2016. Evolution of plant genome architecture. *Genome Biology* 17: 37.

WOO, S.L., A.G. THOMAS, D.P. PESCHKEN, G.G. BOWES, D.W. DOUGLAS, V.L. HARMS, and A.S. MCCLAY. 1991. The biology of Canadian weeds. 99. *Matricaria perforata* Mérat (Asteraceae). *Canadian Journal of Plant Science* 71: 1101–1119.

WOOD, T.E., N. TAKEBAYASHI, M.S. BARKER, I. MAYROSE, P.B. GREENSPOON, and L.H. RIESEBERG. 2009. The frequency of polyploid speciation in vascular plants. *Proceedings of the National Academy of Sciences of the United States of America* 106: 13875–13879.

YAMAUCHI, A., A. HOSOKAWA, H. NAGATA, and M. SHIMODA. 2004. Triploid bridge and role of parthenogenesis in the evolution of autopolyploidy. *The American Naturalist* 164: 101–112.

YANT, L., J.D. HOLLISTER, K.M. WRIGHT, B.J. ARNOLD, J.D. HIGGINS, F.C.H. FRANKLIN, and K. BOMBLIES. 2013. Meiotic adaptation to genome duplication in *Arabidopsis arenosa*. *Current Biology* 23: 2151–2156.

Part B – Case studies

PAPER I

Non-adaptive processes governing early stages of polyploid evolution: Insights from a primary contact zone of relict serpentine *Knautia arvensis* (Caprifoliaceae)

A semi-natural site at serpentine outcrops in the Slavkovský les Mts. where *Knautia* tetraploids co-occur with their diploid progenitors.

Non-adaptive processes governing early stages of polyploid evolution: Insights from a primary contact zone of relict serpentine *Knautia arvensis* (Caprifoliaceae)

Martin Hanzl [1,2,3], Filip Kolář [1,2], Dora Nováková [1] and Jan Suda [1,2]

[1]Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, CZ-128 01 Prague, Czech Republic; and [2]Institute of Botany, Academy of Sciences of the Czech Republic, Zámek 1, CZ-252 43 Průhonice, Czech Republic

[3]Author for correspondence (e-mail: m.hanzl87@gmail.com)

ABSTRACT

Premise of the study**:** Contact zones between polyploids and their diploid progenitors may provide important insights into the mechanisms of sympatric speciation and local adaptation. However, most published studies investigated secondary contact zones where the effects of genome duplication can be confounded by previous independent evolution of currently sympatric cytotypes. We compared genetically close diploid and auto-tetraploid serpentine cytotypes of *Knautia arvensis* (Caprifoliaceae) in a primary contact zone and evaluated the role of adaptive and non-adaptive processes for cytotype coexistence.

Methods**:** DNA flow cytometry was used to determine ploidy distribution at various spatial scales (from across the entire contact zone to microgeographic). Habitat preferences of di- and polyploids were assessed by comparing vegetation composition of nearby ploidy-uniform sites and by recording plant species immediately surrounding both cytotypes in mixed-ploidy plots.

Key Results: Tetraploids considerably outnumbered their diploid progenitors in the contact zone. Both cytotypes were segregated at all investigated spatial scales. This pattern was not driven by ecological shifts as both di- and tetraploids inhabited sites with nearly identical vegetation cover. Certain inter-ploidy niche differentiation was only indicated at the smallest spatial scale; ecologically non-adaptive processes were most likely responsible for this difference.

Conclusions: We conclude that a shift in ecological preferences (i.e., the adaptive scenario) is not necessary for the establishment and evolutionary success of autopolyploid derivatives in primary contact zones. Spatial segregation that would support ploidy coexistence can also be achieved by ecologically non-adaptive processes, including the founder effect, limited dispersal ability, intense clonal growth, and triploid block.

Key words: contact zone; ecological segregation; flow cytometry; minority cytotype exclusion; ploidy coexistence; polyploidy; serpentine; spatial statistics; triploid block

INTRODUCTION

Genome duplication (polyploidy) has played a major role in the evolution of different groups of land plants, ferns and angiosperms in particular (Husband et al., 2013). Recent molecular studies have reshaped our views on the frequency of polyploidization events under natural conditions and revealed the near-ubiquity of genome doubling in the evolutionary history of angiosperms (Fawcett et al., 2013). Polyploidization has been widely acknowledged as the most frequent mode of sympatric speciation (Coyne and Orr, 2004) and might have triggered rapid diversification of certain angiosperm lineages (Soltis et al., 2009). In general, genome duplication is a dynamic and recurrent process (Soltis and Soltis, 1999), which may account for up to 15% of all angiosperm speciation events (Wood et al., 2009). A considerable evolutionary potential of genome duplication likely stems from ploidy-associated changes in the genetic make-up, physiology, anatomy and/or morphology (Levin, 2002; Ramsey and Schemske, 2002; Weiss-Schneeweiss et al., 2013), which may confer novel phenotypes and/or ecological traits with a high adaptive value.

However, the frequency of polyploids observed *in situ* contrasts with theoretical predictions of several mathematical models (Levin, 1975; Fowler and Levin, 1984; Rodríguez, 1996; Rausch and Morgan, 2005) as well as some empirical evidence (Maceira et al., 1993; Husband, 2000; Levin, 2002; Baack, 2005a; Collins et al., 2011), which consider the establishment of a newly arisen polyploid in the population of its diploid progenitor unlikely. Specifically, long-term survival of polyploid derivatives can be hampered by their predominant mating with improper partners, leading to a frequency-dependent selection against the rarer ploidy (i.e., the minority cytotype exclusion principle; Levin, 1975; Husband, 2000).

There are several evolutionary mechanisms that can overcome the disadvantage of being a minority and facilitate the establishment of a new polyploid lineage and subsequent coexistence of different cytotypes (Husband and Sabara, 2004; Baack, 2005b). For instance, a shift from sexual reproduction to clonal growth and/or apomixis can reduce inter-ploidy mating interactions and lead to the increase in abundance of the originally rare cytotype (Thompson and Whitton, 2006; Kao, 2007). Reproductive assurance can also be achieved by autogamy (Barringer, 2007), flowering time divergence (Petit et al., 1997; Maceira et al., 1993; Jersáková et al., 2010), non-random pollen transfer in mixed-ploidy populations (Segraves and Thompson, 1999; Baack, 2005b; Kennedy et al., 2006) or through cryptic breeding barriers operating between pollination and fertilization (Husband et al., 2002). Similarly, sufficient production of unreduced (*2n*) gametes in individuals of a progenitor ploidy and/or frequent recurrent formation of polyploids will help to counteract the minority disadvantage (Husband, 2004; Trávníček et al., 2011a; Suda and Herben, 2013). Genome duplication can often alter ecological behavior of polyploids (Levin, 1983; Ramsey and Schemske, 2002; Ramsey, 2011). If di- and polyploids differ in their habitat preferences, cytotype distribution pattern is largely shaped by ploidy-specific survival in a heterogeneous environment (Li et al., 2004). Distinct habitat preferences of different cytotypes have been reported in many plant groups (e.g., Ehrendorfer, 1980; Johnson et al., 2003; Šafářová and Duchoslav, 2010; Duchoslav et al., 2010; Sonnleitner et al., 2010; Krejčíková et al., 2013) and are considered among the most important factors limiting inter-ploidy interactions (mating, competition for resources, etc.) and facilitating the establishment of newly arisen polyploids (Parisod et al., 2010; Weiss-Schneeweiss et al., 2013).

Evolutionary consequences of genome duplication can be best studied in contact zones of different cytotypes. Two types of ploidy contacts (primary and secondary) are generally recognized according to their evolutionary history (Petit et al., 1999). While primary contact develops as a direct consequence of the emergence of a neopolyploid within a diploid population, secondary contact arises when diploid and polyploid populations meet after some period of spatial segregation. Because, in the latter case, diploid ancestors and polyploid derivatives differentiated in allopatry, their traits can mirror divergent selective pressures acting at spatially isolated sites (Ramsey, 2011). Consequently, phenotypic and/or ecological differences observed between cytotypes in secondary contact zones should be interpreted with caution because the effects of genome duplication can be confounded by independent evolutionary histories of currently coexisting di- and polyploids, and the observed traits are not necessarily the same as they were in early stages of polyploid evolution (i.e., shortly after genome duplication). Critical assessment of factors contributing to the establishment of neopolyploids in sympatric populations with their diploid progenitors therefore requires comparison of genetically close di- and polyploids in primary contact zones. However, the majority of thoroughly investigated contact zones are presumably of secondary origin (e.g., Husband and Sabara, 2004; Duchoslav et al., 2010; Sonnleitner et al., 2010; Trávníček et al., 2011b, 2012; Mráz et al., 2012) while primary zones of ploidy contact are comparatively rare (but see Lumaret et al., 1987; Bretagnolle and Thompson, 1996; Kolář et al., 2009, 2012; Šingliarová et al., 2011). A unique plant system where the incidence of both primary and secondary contact zones was confirmed by molecular markers is *Knautia arvensis* of Central Europe (Kolář et al., 2012). Whereas the contact zone in the Pannonian basin is formed by genetically distinct di- and tetraploids (and represents thus a secondary contact), ploidy mixtures on relict serpentine stands in western Bohemia consist of almost identical genotypes and fit well into the concept of a primary contact. The serpentine plants represent a separate evolutionary lineage well differentiated from their widespread non-serpentine counterparts (Kolář et al., 2012).

Genetic homogeneity of the co-occurring serpentine cytotypes together with the limited extent of the investigated 'microcosm' (6 × 1.5 square kilometers), which allows exhaustive sampling, provide ideal opportunities to address challenging questions surrounding early stages of polyploid evolution. Specifically, we investigated the following questions: (1) What is the spatial structure of diploids and their autotetraploid derivatives across the primary contact zone? (2) What is the fine-scale distribution pattern in mixed-ploidy plots and how can it contribute to a long-term cytotype coexistence? (3) Is the cytotype coexistence primarily governed by ecologically adaptive or non-adaptive mechanisms? While the former refer to the emergence of novel traits as a direct effect of genome duplication (e.g., shift in habitat preferences) the latter refer to inherent traits and/or chance events.

MATERIALS AND METHODS

Study system

Field scabious [*Knautia arvensis* (L.) Coult., Caprifoliaceae] is a rhizomatous, herbaceous perennial native to Europe and western Asia, where it inhabits dry or mesophilous grasslands, forest margins and open forests (Ehrendorfer, 1976; Štěpánek, 1997). Due to its proterandry and gynodioecy, the species is an almost exclusive out-crosser (Ehrendorfer, 1962a), although self-compatibility was confirmed experimentally (Vange, 2002). Two cytotypes with more or less parapatric distribution are recognized in Central Europe (Ehrendorfer, 1962b; Štěpánek, 1997): diploid ($2n = 2x = 20$), referred to as *K. a.* subsp. *pannonica* (Heuffel) O. Schwarz and occurring in the south-eastern part of the distributional range, and tetraploid ($2n = 4x = 40$), referred to as *K. a.* subsp. *arvensis* and occurring in the north-west. In addition, five spatially isolated diploid populations were recorded in Bohemia and northern Bavaria, in areas otherwise inhabited by widespread tetraploids (Štěpánek, 1997; Kaplan, 1998). Molecular analyses showed that these populations represent a distinct evolutionary lineage (Kolář et al., 2012; Rešetnik et al., 2014). They inhabit either relict pine forests (i.e., low-competition sites with species assemblages persisting from the early Holocene) on serpentine bedrock (provisionally treated as *K. a.* subsp. *serpentinicola* Smejkal ined.; Štěpánek, 1982; Kaplan, 1998) or subalpine grasslands in a glacial cirque in the Krkonoše Mts. [described as *K. a.* subsp. *pseudolongifolia* (Szabó) O. Schwarz; Štěpánek, 1989].

Interestingly, detailed flow cytometric investigation revealed a mixture of diploid and tetraploid cytotypes in one relict serpentine site in the Slavkovský les Mts., western Bohemia, the Czech Republic (Kolář et al., 2009). The overall phenotypic similarity, specific ecology (serpentine tolerance) and identical monoploid genome sizes suggested autopolyploid origin of serpentine tetraploids from local diploids and hence a primary contact. This hypothesis was subsequently confirmed by molecular markers (AFLP and cpDNA), which showed considerable genetic similarities between coexisting serpentine di- and tetraploids (Kolář et al., 2012).

Field sampling

Extensive sampling across the entire contact zone was conducted in the Slavkovský les Mts. during 2008–2012. All *Knautia* subpopulations occurring on serpentine bedrock (301 in total) were included to the study, their GPS positions were recorded, and the abundance of *Knautia* plants was assessed (Appendix S1; see Supplemental Data with the online version of this article). Patches distant more than 30 m were treated as separate subpopulations. Leaves from at least 15 *Knautia* individuals per subpopulation (depending on plant abundance) were collected, placed into moist plastic bag and kept in cold until flow cytometric analysis. The distance between sampled plants was at least 0.5 m to decrease the risk of collection of the same genet. ArcGIS 10.0 (ESRI, 2011) was used to draw the distribution map of different cytotypes across the primary contact zone. Historical military-survey map published in 1878 (available on http://oldmaps.geolab.cz) was georeferenced using 30 reference points on a present-day aerial photograph (2nd order polynomial transformation, Total root mean square error: 15.0 m).

In four patches of immediate ploidy contact (representing all available sites with sufficient abundance of both *Knautia* cytotypes), rectangular plots (M1-M4) of appropriate size were laid out to include at least 50 individuals of each majority ($2x$, $4x$) ploidy. Spatial positions of all plants within the plots were recorded using the XY-coordinate system, the plants were classified into three arbitrary life-stage categories (juvenile, vegetative and flowering), and one leaf per plant was collected for ploidy estimation. Based on our field observations, leaf rosettes growing closer than five centimeters were treated as the same genet. In such cases, we counted the total number of rosettes per individual and distinguished the flowering and vegetative ones.

Vegetation surveys

Environmental conditions were characterized with the aid of vegetation samples (phytosociological relevés) in 20 uniformly diploid and 20 uniformly tetraploid subpopulations situated in close proximity. Study plots (5 × 5 m) were laid out in patches with abundant occurrence of *Knautia* plants and the following data were recorded (Appendix S2; see Supplemental Data): relative abundance of all co-occurring species (separately for moss, herb, shrub, and tree layers), cover of each vegetation layer, and slope inclination. Ploidy level of all *Knautia* individuals growing within each vegetation sample was verified by flow cytometry. At microgeographic scale, vegetation surveys were conducted in two mixed-ploidy plots (M1, M2). Presence of all moss and herb species growing within a 15-cm radius of each *Knautia* plant was recorded.

Estimation of DNA ploidy levels

DNA ploidy levels (Suda et al., 2006) were inferred from relative fluorescence intensities of DAPI-stained nuclei by flow cytometry (FCM). Sample preparation generally followed the two-step procedure using Otto buffers (Doležel et al., 2007) and *Bellis perennis* L. (2C = 3.96 pg DNA; Schönswetter et al., 2007a) was selected as an appropriate internal reference standard. Fluorescence of at least 3000 particles was recorded using a Partec PA II flow cytometer (Partec GmbH, Münster, Germany) equipped with a mercury arc lamp as the source of UV excitation light. Up to ten *Knautia* individuals were pooled during the large-scale ploidy screening. Our previous experiments showed that a minority cytotype can be reliably detected using FCM even if it occurs at low frequencies (< 10%). Each plant was analyzed separately if mixed-ploidy samples were found or if the quality of resulting histograms was not sufficient (i.e., coefficient of variation of G_0/G_1 peak of *Knautia* sample above 4%). Karyologically-counted *Knautia* plants (Kolář et al., 2009) were used as reference points for the interpretation of FCM results.

Statistical analyses

Unless stated otherwise, statistical analyses were performed in R ver. 2.15.1 software (R Core Team, 2012). The spatial structure in mixed-ploidy plots was analyzed by the K-function (Ripley, 1977) in the R-package 'spatstat' (Baddeley and Turner, 2005). Each rosette of composed individuals was treated separately and its spatial position was slightly altered (< 5 cm) with respect to the maternal individual to avoid identical coordinates and meet requirements of statistical analyses. The K-function determines the pattern of distribution (clumped, random, regular) by counting the number of neighbors within a given radius r of each individual in the study plot, and comparing the mean number with expected count derived from density of particular individuals in the entire study plot. To depict the spatial patterns at various scales, the K-function was transformed to the L-function (Doležal et al., 2006). On a graph of $L(r)$ vs. r, positive, zero and negative values of the $L(r)$-function indicate clumped, random and regular patterns, respectively, over distances of r. Pair-wise inter-cytotype associations were examined with the bivariate $K_{12}(r)$-function (Cressie, 1993) and visualized with its derived $L_{12}(r)$-function. Positive, neutral and negative associations of cytotypes are assessed by counting only neighbors of the other cytotype within a radius r of each individual. The 95% confidence interval was estimated using a Monte Carlo permutation with 1000 replications (this procedure fixes the distribution pattern of one cytotype while randomly shifting the distribution of the other).

The overall distribution pattern of cytotypes in mixed-ploidy plots was also assessed by the Mantel test (Mantel, 1967) implemented in the R-package 'ade4' (Dray and Dufour, 2007). A pair-wise distance matrix derived from positions of individuals in each plot was compared with a pair-wise binary matrix coding the cytotype identity. Significance level was estimated using a permutation test with 9999 replicates.

Inter-ploidy differences in the proportion of life-stage categories within each mixed-ploidy plot were tested using the chi-square test for homogeneity. This approach allowed an independent comparison of abundances of juvenile, vegetative and flowering individuals of both di- and tetraploids. When assumptions of the chi-square test were not fully met, the results were supplemented by Fisher´s exact test. The Wilcoxon rank sum test was used to evaluate inter-cytotype differences in the production of lateral leaf rosettes.

Ordination techniques were used to assess habitat preferences of different cytotypes (Lepš and Šmilauer, 2003). Prior analyses, relative abundances of species in vegetation samples were log-transformed. Overall similarities in the composition of vegetation samples and/or in accompanying plant assemblages were assessed using the principal component analysis (PCA). The effect of ploidy level was tested by the redundancy analysis (RDA) through random assignment of vegetation samples / plant assemblages to ploidy levels in a Monte Carlo permutation test with 999 replications. Species that selectively accompanied di- or tetraploid *Knautia* cytotypes were identified using a forward selection with a Monte Carlo permutation test (based on a species´ marginal effect in RDA). The analyses were performed in Canoco for Windows ver. 4.5, and CanoDraw ver. 4.0 (ter Braak and Šmilauer, 2002) was used to visualize the results. Each vegetation sample was also characterized by the unweighted mean of the Ellenberg indicator values (EIVs; for light, temperature, continentality, moisture, soil reaction and nutrients) inferred from species composition data using the Juice 7.0 software (Tichý, 2002). EIVs provide estimates of environmental characteristics of the sites inferred from species composition (Ellenberg, 1992). The vegetation samples were then tested for ploidy-specific differences in mean Ellenberg indicator values, species richness, inclination (log-transformed) and cover of particular vegetation layers using the analysis of variance (ANOVA). The results were visualized using Statistica 8.0 (StatSoft, 2007). The vegetation data were supplemented by spatial autocorrelation analysis (Mantel test in the R-package ʻade4ʼ; the significance estimation was based on a permutation test with 9999 replicates). In the contact zone, we compared the matrix of Euclidean distances among cytotype-uniform populations in the two-dimensional PCA ordination space with the distance matrix inferred from geographical locations of corresponding populations. In mixed-ploidy plots, we compared the matrix of Euclidean distances among individuals in the two-dimensional PCA ordination space with the distance matrix inferred from positions of corresponding individuals within the study plots.

RESULTS

Ploidy distribution across the primary contact zone

Four different cytotypes (2*x*, 3*x*, 4*x*, and 6*x*) were detected among 4399 *Knautia* individuals subjected to FCM analysis. Tetraploids were the most common (3278 individuals, ~74.5%), followed by diploids (1119 individuals, ~25.5%). Two minority ploidies (accounting for only 0.05% of sampled individuals) also occurred in the contact zone: one triploid plant grew in otherwise diploid subpopulation (no. 189; Appendix S1) while one hexaploid plant was found in a mixed 2*x*-4*x* subpopulation no. 106 (this record represents the first evidence of hexaploidy in *K. arvensis*). Tetraploid subpopulations (245; = 81.4%) significantly ($\chi^2 = 138$, df = 1, $P < 0.001$) prevailed over their diploid counterparts (45; = 15.0%). Sympatric growth of both majority cytotypes was recorded in 11 subpopulations (3.6%). The two majority cytotypes showed contrasting patterns of distribution: whereas tetraploid subpopulations were distributed across the entire serpentine body, diploid subpopulations were largely restricted to three isolated patches, which corresponded to historically continuous forest areas and were therefore less affected by man (Fig. 1). Mixed-ploidy subpopulations mostly occurred near the contact of 2*x* and 4*x* cytotype-uniform subpopulations, the only exceptions being two subpopulations (nos. 154 and 243; Appendix S1), which lacked any pure diploid subpopulation in close proximity

Ploidy distribution at microgeographic scale

The four thoroughly investigated mixed-ploidy plots (M1-M4) considerably differed both in plant abundances and proportions of di- and tetraploids (Tab. 1). Most *Knautia* plants showed intense clonal growth and consisted of several presumably interconnected rosettes. In three out of the four mixed-ploidy plots, diploids produced significantly greater number of lateral rosettes (Tab. 1). Mantel test revealed non-random distribution of di- and tetraploids in all plots (Tab. 1). Deeper insight into the spatial structure was provided by the *L(r)* function, which showed positive associations of individuals of the same ploidy and negative associations of di- and tetraploids. While spatial correlations were significant for the entire range of distances in the two plots with more abundant *Knautia* plants (r = 0–250 cm and 0–300 cm in M1 and M2, respectively; Fig. 2, Appendix S3; see Supplemental Data), only associations at distances above 70 cm were significant in the two remaining plots (M3, M4; Appendices S4, S5; see Supplemental Data).

The same pattern (i.e., positive intra-cytotype and negative inter-cytotype associations) was also documented for each life-stage category in the two *Knautia*-rich plots (M1 and M2; Appendices S6, S7; see Supplemental Data). In addition, sympatric di- and tetraploids differed in a relative frequency of individual life-stages. These differences were significant in three of the four studied plots (M2-M4) and marginally non-significant in the plot M1 (Tab. 1). In comparison to their diploid counterparts, tetraploids usually showed lower proportions of flowering individuals and juvenile plants (Fig. 3).

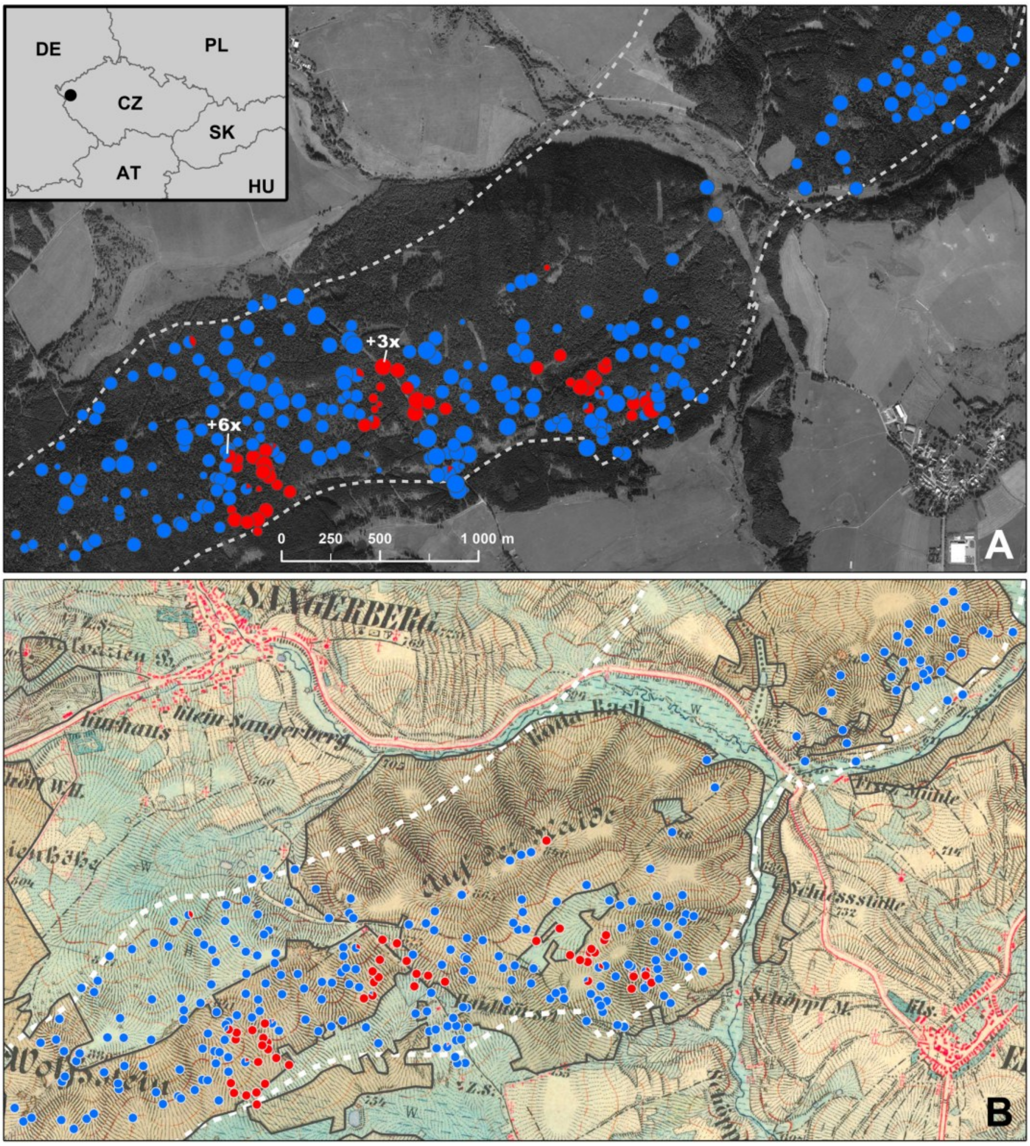

Fig. 1. Recent distribution of diploid (red), tetraploid (blue) and mixed-ploidy (pie charts) subpopulations in the primary contact zone of *Knautia arvensis* on a serpentine outcrop in western Bohemia (Central Europe). The dashed lines delimit the boundary of the serpentine area. (A) Current extent of forest on an aerial photograph from 2011. Symbol size corresponds to plant abundance; the presence of two minority cytotypes (3*x*, 6*x*) is also indicated. (B) Historical map from 1878, showing the reduced extent of forests (brown parts with thick contours) that were locally replaced by pastures (depicted in blue). Note that diploids are almost exclusively confined to continuously forested sites.

Table 1. Plant abundance, cytotype proportions, intensity of clonal growth, spatial pattern of cytotype distribution and life-stage composition of *Knautia arvensis* cytotypes occurring in the four mixed-ploidy plots (M1-M4). *P*-values below 0.05 highlighted in bold. See the text for details.

Plot	Total number of *Knautia* individuals	2*x* [%]	4*x* [%]	Mean no. of lateral rosettes				Spatial associations of cytotypes		Life-stage proportions of cytotypes		
				2*x*	4*x*	*W*	*P*	r_M	*P*	X^2	df	*P*
M1	475	21.1	78.9	2.3	1.3	25204	**< 0.001**	0.517	**< 0.001**	5.69	2	0.058
M2	291	66.0	34.0	2.8	1.4	11643	**0.001**	0.803	**< 0.001**	14.57	2	**< 0.001**
M3	48	50.0	50.0	1.4	0.7	388.5	**0.029**	0.403	**< 0.001**	6.29	2	**0.043**
M4	98	71.4	28.6	1.5	1.0	1043	0.606	0.406	**< 0.001**	11.91	2	**0.003 (0.004)**[a]

Note: [a] *P*-value of Fisher´s exact test in parentheses.

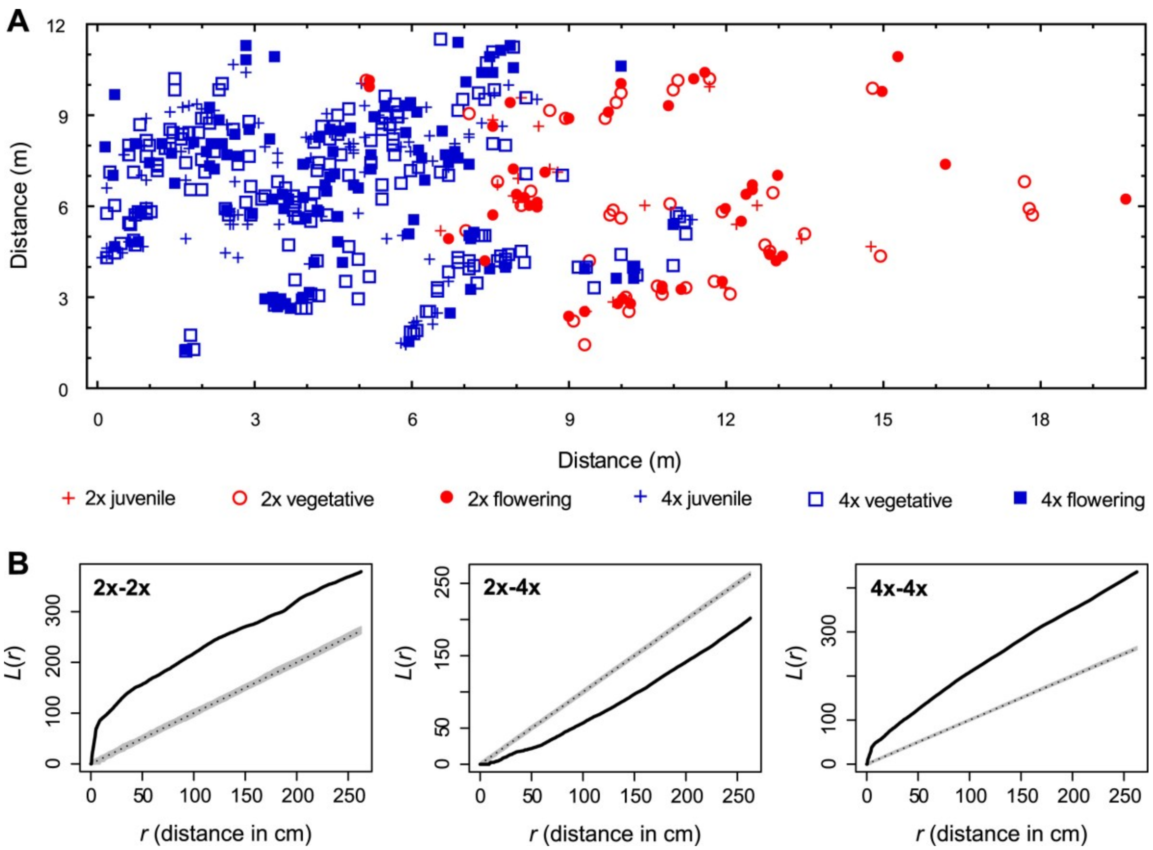

Fig. 2. Spatial positions of all Knautia individuals (divided into three life-stages) in a mixed-ploidy plot M1 (A) and corresponding pair-wise inter- and intra-cytotype spatial associations (B). Expected values of the L(r) function are shown by the dotted line, 95% confidence interval shaded in grey. Values higher or lower than confidence limits indicate positive or negative associations, respectively.

Ecological characteristics of cytotypes

Diploid and tetraploid subpopulations did not differ in the composition of accompanying vegetation as demonstrated both by the intermingled position of vegetation samples in PCA (Fig. 4) and by a permutation test in RDA ($P = 0.333$). Similarly, no significant ploidy-specific differences were detected in selected environmental characteristics of studied *Knautia* subpopulations (Tab. 2), including inclination, overall species richness, relative cover of particular vegetation layers and/or mean Ellenberg indicator values (Appendix S8; see Supplemental Data). Vegetation similarity was independent of the spatial position of samples (non-significant results of spatial autocorrelation analysis: $r_M = 0.041$, $P = 0.18$).

A certain niche differentiation was indicated at the microspatial scale in two mixed-ploidy plots, comprising a total of 136 diploid and 247 tetraploid *Knautia* plants. The RDA analysis revealed significant differences in species composition immediately surrounding 2*x* and 4*x* *Knautia* in both M1 ($P = 0.001$) and M2 ($P = 0.001$) plots. Species significantly associated with 2*x* and 4*x* *Knautia* plants are summarized in Table 3. The distance matrix of *Knautia* individuals was significantly correlated with the resemblance matrix of accompanying vegetation in both M1 ($r_M = 0.195$, $P < 0.001$) and M2 ($r_M = 0.519$, $P < 0.001$) plots. PCA diagrams of immediately surrounding vegetation showed that *Knautia* diploids fall within the variation recorded in their tetraploid counterparts in the M1 plot (Fig. 5A) while both cytotypes were segregated in the M2 plot (Fig. 5B).

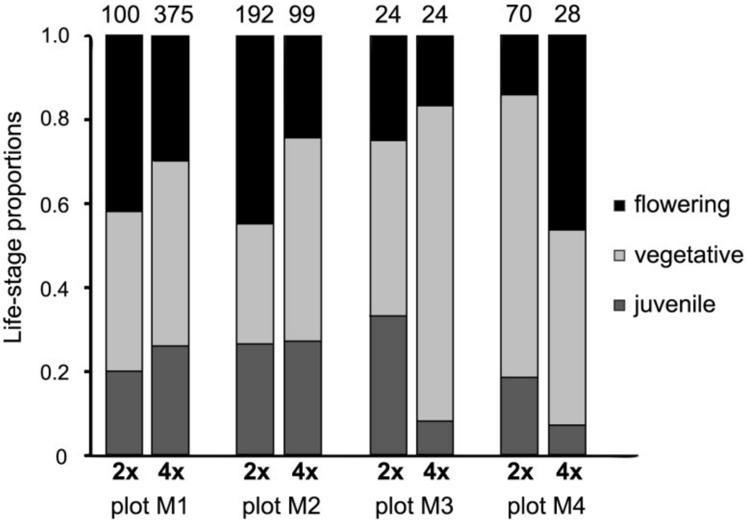

Fig. 3. Proportions of particular life-stages of diploid and tetraploid *Knautia* plants in four thoroughly sampled mixed-ploidy plots. The total number of individuals is shown above each bar.

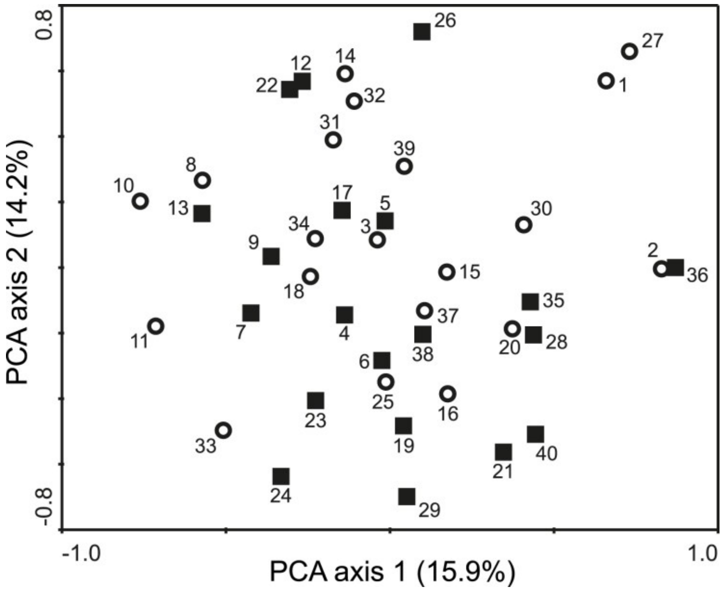

Fig. 4. Unconstrained ordination (principal component analysis) of vegetation samples (vascular plants, mosses) in 40 ploidy-uniform *Knautia* subpopulations (diploid: open circles, tetraploid: solid squares). Numbers correspond to the codes of vegetation samples (see Appendix S2).

Table 2. Results of ANOVA tests for ploidy-specific differences in environmental characteristics of cytotype uniform subpopulations from the primary contact zone of *Knautia arvensis*.

Environmental variable	Analysis of variance		
	F	df	*P*
EIV light	0.69	1, 38	0.411
EIV temperature	1.11	1, 38	0.299
EIV continentality	0.01	1, 38	0.945
EIV moisture	2.13	1, 38	0.152
EIV soil reaction	0.55	1, 38	0.465
EIV nutrients	0.01	1, 38	0.923
Rel. cov. of moss layer	0.15	1, 38	0.704
Rel. cov. of herb layer	0.92	1, 38	0.343
Rel. cov. of tree layer	0.84	1, 38	0.365
Overall species richness	1.67	1, 38	0.204
Slope inclination	0.26	1, 38	0.610

Notes: EIV = Ellenberg indicator values (provide estimates of environmental characteristics of the sites inferred from species composition data); Rel. cov. = relative coverage.

Table 3. List of accompanying species significantly associated with *Knautia* diploids (2*x*) and/or tetraploids (4*x*) in the two mixed-ploidy plots with abundant *Knautia* plants. The lack of ploidy-specific association (*n.s.*) and the absence of a species (-) in a particular plot are also shown.

Plant species	Plot	
	M1	M2
Agrostis capillaris	*n.s.*	2*x*
Cerastium alsinifolium	2*x*	4*x*
Deschampsia cespitosa	*n.s.*	2*x*
Festuca ovina	*n.s.*	2*x*
Festuca rubra	-	4*x*
Hylocomium splendens	2*x*	-
Lathyrus pratensis	*n.s.*	2*x*
Pimpinella major	4*x*	-
Pleurozium schreberi	4*x*	*n.s.*
Potentilla erecta	*n.s.*	2*x*
Pteridium aquilinum	-	4*x*
Ranunculus acris	2*x*	4*x*
Rhytidiadelphus squarrosus	4*x*	*n.s.*
Silene vulgaris	2*x*	-
Tephroseris crispa	-	4*x*

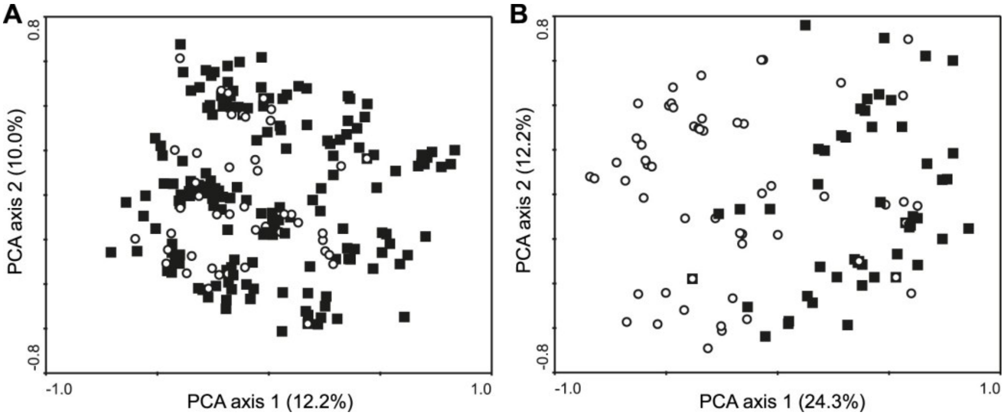

Fig. 5. Unconstrained ordination (principal component analysis) of species immediately accompanying diploid (open circles) and tetraploid (solid squares) *Knautia* plants in the two mixed-ploidy plots M1 (A) and M2 (B). The degree of inter-cytotype microhabitat segregation at both sites most likely reflects local spatial clustering of di- and tetraploids (cf. Fig. 2A and Appendix S3; see Supplemental Data).

DISCUSSION

Our study is a pioneering attempt to explore patterns and processes in a primary contact zone of diploids and their local autotetraploid derivatives. In particular, we focused on the role of ecologically adaptive *versus* non-adaptive processes in the establishment of neo-polyploids and their subsequent coexistence with diploid progenitors.

Spatio-temporal structure of the primary contact zone

Serpentine 2*x*-4*x* populations of *K. arvensis* in western Bohemia represent one of the very few examples of a primary contact of different cytotypes that was supported by several independent pieces of evidence, including morphology (the overall phenotypic similarity), ecology (exclusive growth on serpentine soils), and genetics (genetic homogeneity of coexisting cytotypes) (Kaplan, 1998; Kolář et al., 2009, 2012). Specific edaphic conditions of serpentine island-like habitats have been shown to contribute to local evolution (Kruckeberg, 1954; Brady et al., 2005) and most likely also preserved distinct *Knautia* genotypes whose evolutionary history was more-or-less independent of the surrounding biota. A small extent of the contact zone together with its easy delimitation provided unique opportunities for detailed investigation of cytotype distribution patterns and assessment of potential ploidy-specific differences in ecological requirements.

Despite their presumably low initial abundance, tetraploids currently considerably prevail across the studied serpentine locality and nearly three-fold outnumber their putative diploid progenitors. The present distribution of diploids seems to largely mirror environmental conditions existing in the region in the past. Disregarding few diploid plants, which may represent recent introductions due to human activities, diploids are now restricted to three isolated patches, which coincide with historically forested sites (Fig. 1B). On the contrary, tetraploids currently occur at sites where either forests or pastures were reconstructed historically. These data indicate either wider ecological tolerance of tetraploids (ability to survive in both shaded and open habitats) or their better colonization abilities (successful re-colonization of once open sites). In general, polyploids were found to be more efficient colonizers of deglaciated regions (Brochmann et al., 2004) and they also prevail among invasive plants (te Beest et al., 2012). Nonetheless, experimental study comparing the performance of di- and tetraploid *Knautia* plants is required to elucidate the mechanisms behind the current dominance of tetraploids in the primary contact zone.

Considering the assumed temporal reversal in the frequency of di- and tetraploids, a question arises about the time scale of this process (i.e., the age of the contact zone). Several independent pieces of evidence indicate that the contact zone is most likely of postglacial origin (i.e., a few thousand years old). It is unlikely that *Knautia arvensis* could have survived the glacial periods *in situ* on serpentine outcrops as ecological conditions of mid-altitude habitats in western Bohemia (i.e., prevailing steppe-tundra vegetation; Hahne, 1992; Fletcher et al., 2010) were unsuitable for this species. Similarly, the plants can hardly be counted among recent immigrants (within the time span of a few hundreds of years) because (i) they inhabit relict sites with specific edaphic conditions and (ii) the populations are genetically diverse with a high proportion of private genetic markers (Kolář et al., 2012). In addition, the colonization of the entire serpentine body by originally rare (neo)tetraploids was likely slow and required sufficient time due to limited dispersal abilities of *Knautia* achenes, which are adapted to ant dispersal (presence of an elaiosome; Štěpánek, 1997) with mean transport distances of about two meters (Gómez and Espadaler, 2013). A relatively young age of a contact zone of serpentine *Knautia* indicates that polyploidization can quite rapidly shape population structures not only in invasive allopolyploids (e.g., *Spartina*: Ainouche et al., 2009; *Tragopogon*: Novak et al., 1991) but also in native autopolyploids inhabiting stable relict localities.

Lack of inter-ploidy niche differentiation

Cytotype distribution in many diploid-polyploid contact zones was found to be shaped by environmental heterogeneity (e.g., Rothera and Davy, 1986; Lumaret et al., 1987; Felber-Girard et al., 1996; Johnson et al., 2003, Hülber et al., 2009). Consequently, niche segregation between coexisting cytotypes is one of the most commonly cited factors facilitating the establishment of newly arisen polyploids (Parisod et al., 2010; Weiss-Schneeweiss et al., 2013).

In contrast, our study did not find any ecological sorting of diploid and tetraploid *Knautia* subpopulations in the primary contact zone. Not only was there a lack of habitat segregation in the ordination of associated vegetation (PCA) but the effect of ploidy level was non-significant also in the direct permutation-based tests in the constrained ordination (RDA). Similarly, the sites occupied by di- *versus* polyploids showed great similarities in all recorded parameters, including site inclination, the number of co-occurring species, relative cover of particular vegetation layers, and/or mean Ellenberg indicator values. Previously, the lack of habitat differentiation among coexisting cytotypes was reported for instance in *Andropogon gerardii* (Keeler, 1992) or *Solidago altissima* (Halverson et al., 2008). We admit that the total number of 40 vegetation samples (20 diploid and 20 tetraploid) in our study is relatively low, however, it cannot be increased because no other sufficiently abundant diploid subpopulations were present in the study area. It should also be noted that despite the limited extent of the investigated serpentine outcrop, individual vegetation samples were independent (i.e., the dataset did not contain any pseudoreplications) as indicated by non-significant results of the Mantel test.

We observed significant inter-ploidy differences in associated floristic composition only at the finest spatial scale (within the two mixed-ploidy plots). However, these differences most likely stemmed from ecologically non-adaptive processes and do not support a shift in ecological requirements of coexisting di- and autotetraploids. First, individuals of the same ploidy level were significantly spatially autocorrelated, which can well explain intra-ploidy similarities and inter-ploidy differences in vegetation composition immediately surrounding *Knautia* plants. In addition, none of the 15 accompanying plant species that were significantly associated with either di- or tetraploids showed a consistent pattern. Moreover, two species significantly associated with diploids in the M1 plot but with tetraploids in the M2 plot (see Tab. 3). Our observations demonstrate that contrasting results about niche segregation can be obtained at different geographic scales. Potential ploidy-specific ecological requirements of sympatric cytotypes should therefore be addressed at several different spatial scales, from global to microgeographic, although this has only rarely been achieved, for example in *Senecio carniolicus* (Hülber et al., 2009; Sonnleitner et al., 2010) or *Allium oleraceum* (Duchoslav et al., 2010; Šafářová and Duchoslav, 2010).

When ploidy-driven shifts in ecological requirements of coexisting cytotypes are interpreted, the origin of the contact zone (i.e., whether primary or secondary) can play a pivotal role and should therefore be carefully considered. Specifically, one should take into account that habitat preferences of particular cytotypes in secondary contact zones evolved in allopatry, possibly under divergent selective pressures. Ecological sorting observed in secondary zones is therefore not necessarily ploidy-driven but can merely reflect independent evolutionary histories of different cytotypes, which regained contact after some period of geographic isolation (Mráz et al., 2012). Consequently, the role of niche segregation in early stages of polyploid evolution (e.g., for the avoidance of minority cytotype exclusion and successful neopolyploid establishment) can hardly be assessed from recent observations of secondary contact zones. Given the majority of contact zones is presumably of secondary origin (Petit et al., 1999), the importance of ecological sorting for the establishment of new polyploid derivatives can possibly be overestimated. On the contrary, long-term independent evolution of coexisting cytotypes is avoided in primary contact zones and close genetic relationships between diploid ancestors and polyploid derivatives allow the effects of genome duplication *per se* to be more reliably assessed. Primary contacts better mirror conditions existing in early stages of polyploid evolution and provide valuable insights into the forces governing the establishment of a newly arisen polyploid in sympatry with its dominant diploid progenitor.

Our data collectively demonstrate that neither genome duplication nor subsequent selection on the serpentine cytotype(s) of *K. arvensis* did alter ecological requirements of plants and prevented neo-tetraploids from interactions with their ancestral diploid race. It is therefore unlikely that ecologically-adaptive processes played a major role in polyploid establishment in the investigated primary contact zone.

Non-adaptive processes contributing to spatial segregation

Considering the lack of inter-ploidy niche differentiation, alternative mechanisms governing the spatial segregation and contributing to polyploid establishment and subsequent cytotype coexistence should be sought. There are several non-adaptive mechanisms that can influence the fate of a new polyploid and lead to non-random cytotype distribution in contact zones, including the intensity of clonal reproduction (Keeler, 2004; Kao, 2007), seed dispersability (Baack, 2005b) and/or the strength of selection against inter-ploidy hybrids (van Dijk et al., 1992; Hardy et al., 2000). All of these mechanisms likely act in concert in mixed-ploidy serpentine populations of *K. arvensis*. The founder effect, reducing the chance of a new subpopulation to be founded by both di- and tetraploid achenes, together with limited seed dispersal (Štěpánek, 1997) and vigorous clonal growth (mean 2.7 lateral rosettes per plant) support clumped distribution of same-ploidy individuals and the incidence of ploidy-uniform patches. Although diploid plants exhibited greater number of lateral rosettes at all but one study sites, the increased clonality did not enhance their colonization potential. Spatial segregation of *Knautia* di- and tetraploids was observed from regional (i.e., the prevalence of single-cytotype subpopulations within the entire serpentine body; Fig. 1A) to microgeographic scales (i.e., aggregation of identical cytotypes in mixed-ploidy plots; Table 1, Fig. 2, Appendices S3, S4, S5). There were only few places where both cytotypes came into immediate spatial contact (at the scale of decimeters to centimeters). Non-random cytotype distribution is among the principal non-adaptive mechanisms driving cytotype coexistence (Husband and Sabara, 2004) and was previously documented in numerous plant species with ploidy heterogeneity (e.g., Baack, 2004; Schönswetter et al., 2007b; Šafářová and Duchoslav, 2010; Trávníček et al., 2011a, b).

Spatial segregation of different cytotypes can enhance the stability of ploidy coexistence by increasing the rate of assortative mating in cytotype-uniform clumps (Kennedy et al., 2006) and/or by reducing inter-cytotype competition for resources (Levin, 2002). Moreover, there seems to be also certain intrinsic breeding barriers between *Knautia* diploids and tetraploids, additional to the effect of spatial segregation, as indicated by the lack of triploid hybrids in mixed-ploidy plots. Despite intensive ploidy screening (more than 4000 cytotyped plants) we found only one triploid individual in otherwise diploid subpopulation (representing the third record of triploidy in the *K. arvensis* agg. under natural conditions; see Kolář et al., 2009) and one hexaploid individual (previously unknown) growing close to tetraploids in a mixed 2*x*-4*x* subpopulation. Obviously, both minority cytotypes originated via syngamy of reduced and unreduced gametes of respective parental plants while inter-ploidy hybridization was not supported. Strong triploid block in *Knautia* therefore seems to stabilize cytotype coexistence even in zones of immediate ploidy contact.

We are aware that our approach adopted for spatial analyses in mixed-ploidy plots (i.e., treating all rosettes of composed individuals as separate entities) can overestimate the degree of inter-ploidy segregation. Nonetheless, we believe that this design better reflects field conditions because it offers more precise estimates of local densities of *Knautia* ramets and is more relevant for assessing reproductive interactions. In addition, our method could possibly bias the results at the finest spatial scale, however, different cytotypes in mixed-ploidy plots were segregated at all investigated spatial scales. Finally, spatial analyses of putative genets (i.e., treating composed *Knautia* individuals as one unit) yielded highly comparable results (data not shown). We should also mention that although the detailed distribution pattern was only studied in four mixed-ploidy plots, these represented all available sites with sufficient numbers of plants, and any further sampling was not possible.

Unlike previous studies aimed at assessing cytotype distribution at fine spatial scales (e.g., Baack, 2004; Kao and Parker, 2010; Šafářová and Duchoslav, 2010; Trávníček et al., 2011b; Mráz et al., 2012), we not only recorded spatial positions of all individuals in mixed-ploidy plots but also distinguished main life-stages (juvenile, vegetative and flowering plants). This approach allowed us to develop hypotheses about temporal changes in cytotype frequencies. In three out of four microspatial plots, coexisting di- and tetraploids significantly differed in proportions of individual life-stage categories, which may indicate the dynamic nature of mixed-ploidy populations and generally unstable coexistence of 2*x* and 4*x* *Knautia* plants on a longer time scale. Considering the large variation in abundances and life-stage distributions of the cytotypes in the four thoroughly sampled plots, both diploids and tetraploids can be the first to vanish from mixed-ploidy sites. However, a detailed demographic study and the assessment of ploidy-specific fitness and competition abilities is required to get more holistic insight into the dynamics of contact zones of *K. arvensis*.

Conclusions

The present study addresses patterns and processes associated with the establishment and evolutionary trajectories of novel polyploid lineages in sympatric populations with their direct diploid progenitor (i.e., within a primary contact zone). Our microcosm involved genetically close di- and tetraploids inhabiting an insular-like site with specific edaphic conditions (serpentine outcrop), which avoided potential bias due to independent evolutionary histories of currently co-occurring cytotypes. We found segregation of coexisting di- and tetraploids of *Knautia arvensis* at all investigated spatial scales (from across the entire serpentine body to microspatial), however, this segregation was not driven by an ecological shift following genome duplication. The spatial cytotype structure was likely shaped by non-adaptive processes, including the founder effect, limited seed dispersal, and/or plant longevity/clonality. Ploidy coexistence was also stabilized by strong inter-ploidy breeding barriers, which limited mating interactions even in zones of immediate ploidy contact. Future work should elucidate the role of demographic parameters and ploidy-specific fitness.

The main message of our study is that niche differentiation is not a necessary prerequisite for a long-term coexistence of different ploidy levels and spatial segregation in primary contact zones can also be achieved by other mechanisms. Polyploid derivatives can successfully establish and spread even if they share habitat preferences with their diploid progenitors. Possibly, the importance of niche differentiation for polyploid establishment can currently be exaggerated due to the predominance of data from secondary contact zones.

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LITERATURE CITED

Ainouche, M. L., P. M. Fortune, A. Salmon, C. Parisod, M. A. Grandbastien, K. Fukunaga, M. Ricou, and M. T. Misset. 2009. Hybridization, polyploidy and invasion: lessons from *Spartina* (Poaceae). *Biological Invasions* 11: 1159-1173.

Baack, E. J. 2004. Cytotype segregation on regional and microgeographic scales in snow buttercups (*Ranunculus adoneus* : Ranunculaceae). *American Journal of Botany* 91: 1783-1788.

Baack, E. J. 2005a. Ecological factors influencing tetraploid, establishment in snow buttercups (*Ranunculus adoneus*, Ranunculaceae): minority cytotype exclusion and barriers to triploid formation. *American Journal of Botany* 92: 1827-1835.

Baack, E. J. 2005b. To succeed globally, disperse locally: effects of local pollen and seed dispersal on tetraploid establishment. *Heredity* 94: 538-546.

Baddeley, A., and R. Turner. 2005. spatstat: an R package for analyzing spatial point patterns. *Journal of Statistical Software* 12: 1-42.

Barringer, B. C. 2007. Polyploidy and self-fertilization in flowering plants. *American Journal of Botany* 94: 1527-1533.

Brady, K. U., A. R. Kruckeberg, and H. D. Bradshaw. 2005. Evolutionary ecology of plant adaptation to serpentine soils. *Annual Review of Ecology, Evolution, and Systematics* 36: 243-266.

Bretagnolle, F., and J. D. Thompson. 1996. An experimental study of ecological differences in winter growth between sympatric diploid and autotetraploid *Dactylis glomerata*. *Journal of Ecology* 84: 343-351.

Brochmann, C., A. K. Brysting, I. G. Alsos, L. Borgen, H. H. Grundt, A. C. Scheen, and R. Elven. 2004. Polyploidy in arctic plants. *Biological Journal of the Linnean Society* 82: 521-536.

Collins, A. R., R. Naderi, and H. Mueller-Schaerer. 2011. Competition between cytotypes changes across a longitudinal gradient in *Centaurea stoebe* (Asteraceae). *American Journal of Botany* 98: 1935-1942.

Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer Associates, Sunderland, Massachusetts, USA.

Cressie, N. A. C. 1993. Statistics for spatial data. Wiley, New York, New York, USA.

Doležal, J., M. Šrutek, T. Hara, A. Sumida, and T. Penttilä. 2006. Neighborhood interactions influencing tree population dynamics in nonpyrogenous boreal forest in northern Finland. *Plant Ecology* 185: 135-150.

Doležel, J., J. Greilhuber, and J. Suda. 2007. Estimation of nuclear DNA content in plants using flow cytometry. *Nature Protocols* 2: 2233-2244.

Dray, S., and A. B. Dufour. 2007. The ade4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software* 22: 1-20.

Duchoslav, M., L. Šafářová, and F. Krahulec. 2010. Complex distribution patterns, ecology and coexistence of ploidy levels of *Allium oleraceum* (Alliaceae) in the Czech Republic. *Annals of Botany* 105: 719-735.

Ehrendorfer, F. 1962a. Beiträge zur Phylogenie der Gattung *Knautia* (Dipsacaceae). I. Cytologische Grundlagen und allgemeine Hinweise. *Österreichische Botanische Zeitschrift* 109: 276-343.

Ehrendorfer, F. 1962b. Cytotaxonomische Beiträge zur Genese der mitteleuropäischen Flora und Vegetation. *Berichte der Deutschen Botanischen Gesellschaft* 75: 137-152.

Ehrendorfer, F. 1976. *Knautia* L. *In* T. Tutin [ed.], Flora Europaea, vol. 4, 60-67. Cambridge University Press, Cambridge, UK.

Ehrendorfer, F. 1980. Polyploidy and distribution. *In* W. H. Lewis [ed.], Polyploidy: Biological Relevance, 45-60. Plenum Press, New York, New York, USA.

Ellenberg, H. 1992. Zeigerwerte der Pflanzen in Mitteleuropa, 3rd ed. E. Goltze, Göttingen, Germany.

ESRI. 2011. ArcGIS Desktop, version 10.0. Environmental Systems Research Institute, Redlands, California, USA.

Fawcett, J. A., Y. Van de Peer, and S. Maere. 2013. Significance and biological consequences of polyploidization in land plant evolution. *In* I. J. Leitch, J. Greilhuber, J. Doležel, and J. F. Wendel [eds.], Plant Genome Diversity, vol. 2, 277-293. Springer, New York, New York, USA.

Felber-Girard, M., F. Felber, and A. Buttler. 1996. Habitat differentiation in a narrow hybrid zone between diploid and tetraploid *Anthoxanthum alpinum*. *New Phytologist* 133: 531-540.

Fletcher, W. J., M. F. S. Goni, J. R. M. Allen, R. Cheddadi, N. Combourieu-Nebout, B. Huntley, I. Lawson, et al. 2010. Millennial-scale variability during the last glacial in vegetation records from Europe. *Quaternary Science Reviews* 29: 2839-2864.

Fowler, N. L., and D. A. Levin. 1984. Ecological constraints on the establishment of a novel polyploid in competition with its diploid progenitor. *American Naturalist* 124: 703-711.

Gómez, C., and X. Espadaler. 2013. An update of the world survey of myrmecochorous dispersal distances. *Ecography* 36: 1193-1201.

Hahne, J. 1992. Untersuchungen zur spät- und postglazialen Vegetationsgeschichte im nordöstlichen Bayern (Bayerisches Vogtland, Fichtelgebirge, Steinwald). *Flora* 187:169-200.

Halverson, K., S. B. Heard, J. D. Nason, and J. O. Stireman. 2008. Origins, distribution, and local co-occurrence of polyploid cytotypes in *Solidago altissima* (Asteraceae). *American Journal of Botany* 95: 50-58.

Hardy, O. J., S. Vanderhoeven, M. de Loose, and P. Meerts. 2000. Ecological, morphological and allozymic differentiation between diploid and tetraploid knapweeds (*Centaurea jacea*) from a contact zone in the Belgian Ardennes. *New Phytologist* 146: 281-290.

Hülber, K., M. Sonnleitner, R. Flatscher, A. Berger, R. Dobrovsky, S. Niessner, T. Nigl, et al. 2009. Ecological segregation drives fine-scale cytotype distribution of *Senecio carniolicus* in the Eastern Alps. *Preslia* 81: 309-319.

Husband, B. C. 2000. Constraints on polyploid evolution: a test of the minority cytotype exclusion principle. *Proceedings of the Royal Society B-Biological Sciences* 267: 217-223.

Husband, B. C. 2004. The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. *Biological Journal of the Linnean Society* 82: 537-546.

Husband, B. C., and H. A. Sabara. 2004. Reproductive isolation between autotetraploids and their diploid progenitors in fireweed, *Chamerion angustifolium* (Onagraceae). *New Phytologist* 161: 703-713.

Husband, B. C., D. W. Schemske, T. L. Burton, and C. Goodwillie. 2002. Pollen competition as a unilateral reproductive barrier between sympatric *Chamerion angustifolium*. *Proceedings of the Royal Society, B, Biological Sciences* 269: 2565-2571.

Husband, B. C., S. J. Baldwin, and J. Suda. 2013. The incidence of polyploidy in natural plant populations: major patterns and evolutionary processes. *In* I. J. Leitch, J. Greilhuber, J. Doležel, and J. F. Wendel [eds.], Plant Genome Diversity, vol. 2, 255-276. Springer, New York, New York, USA.

Jersáková, J., S. Castro, N. Sonk, K. Milchreit, I. Schödelbauerová, T. Tolasch, and S. Dötterl. 2010. Absence of pollinator-mediated premating barriers in mixed-ploidy populations of *Gymnadenia conopsea* s.l. (Orchidaceae). *Evolutionary Ecology* 24: 1199-1218.

Johnson, M. T. J., B. C. Husband, and T. L. Burton. 2003. Habitat differentiation between diploid and tetraploid *Galax urceolata* (Diapensiaceae). *International Journal of Plant Sciences* 164: 703-710.

Kao, R. H. 2007. Asexuality and the coexistence of cytotypes. *New Phytologist* 175: 764-772.

Kao, R. H., and I. M. Parker. 2010. Coexisting cytotypes of *Arnica cordifolia*: morphological differentiation and local-scale distribution. *International Journal of Plant Sciences* 171: 81-89.

Kaplan, Z. 1998. Relict serpentine populations of *Knautia arvensis* s. l. (Dipsacaceae) in the Czech Republic and an adjacent area of Germany. *Preslia* 70: 21-31.

Keeler, K. H. 1992. Local polyploid variation in the native prairie grass *Andropogon gerardii*. *American Journal of Botany* 79: 1229-1232.

Keeler, K. H. 2004. Impact of intraspecific polyploidy in *Andropogon gerardii* (Poaceae) populations. *American Midland Naturalist* 152: 63-74.

Kennedy, B. F., H. A. Sabara, D. Haydon, and B. C. Husband. 2006. Pollinator-mediated assortative mating in mixed ploidy populations of *Chamerion angustifolium* (Onagraceae). *Oecologia* 150: 398-408.

Kolář, F., T. Fér, M. Štech, P. Trávníček, E. Dušková, P. Schönswetter, and J. Suda. 2012. Bringing together evolution on serpentine and polyploidy: spatiotemporal history of the diploid-tetraploid complex of *Knautia arvensis* (Dipsacaceae). *Plos One* 7: e39988.

Kolář, F., M. Štech, P. Trávníček, J. Rauchová, T. Urfus, P. Vít, M. Kubešová, et al. 2009. Towards resolving the *Knautia arvensis* agg. (Dipsacaceae) puzzle: primary and secondary contact zones and ploidy segregation at landscape and microgeographic scales. *Annals of Botany* 103: 963-974.

Krejčíková, J., R. Sudová, M. Lučanová, P. Trávníček, T. Urfus, P. Vít, H. Weiss-Schneeweiss, et al. 2013. High ploidy diversity and distinct patterns of cytotype distribution in a widespread species of *Oxalis* in the Greater Cape Floristic Region. *Annals of Botany* 111: 641-649.

Kruckeberg, A. R. 1954. The ecology of serpentine soils. III. Plant species in relation to serpentine soils. *Ecology* 35: 267-274.

Lepš, J., and P. Šmilauer. 2003. Multivariate analysis of ecological data using CANOCO. Cambridge University Press, Cambridge, UK.

Levin, D. A. 1975. Minority cytotype exclusion in local plant populations. *Taxon* 24: 35-43.

Levin, D. A. 1983. Polyploidy and novelty in flowering plants. *American Naturalist* 122: 1-25.

Levin, D. A. 2002. The role of chromosomal change in plant evolution. Oxford University Press, Oxford, UK.

Li, B. H., X. M. Xu, and M. S. Ridout. 2004. Modelling the establishment and spread of autotetraploid plants in a spatially heterogeneous environment. *Journal of Evolutionary Biology* 17: 562-573.

Lumaret, R., J. L. Guillerm, J. Delay, A. A. L. Loutfi, J. Izco, and M. Jay. 1987. Polyploidy and habitat differentiation in *Dactylis glomerata* L. from Galicia (Spain). *Oecologia* 73: 436-446.

Maceira, N. O., P. Jacquard, and R. Lumaret. 1993. Competition between diploid and derivative autotetraploid *Dactylis glomerata* L. from Galicia. Implications for the establishment of novel polyploid populations. *New Phytologist* 124: 321-328.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27: 209-220.

Mráz, P., S. Španiel, A. Keller, G. Bowmann, A. Farkas, B. Šingliarová, R. P. Rohr, et al. 2012. Anthropogenic disturbance as a driver of microspatial and microhabitat segregation of cytotypes of *Centaurea stoebe* and cytotype interactions in secondary contact zones. *Annals of Botany* 110: 615-627.

Novak, S. J., D. E. Soltis, and P. S. Soltis. 1991. Ownbey´s Tragopogons: 40 years later. *American Journal of Botany* 78: 1586-1600.

Parisod, C., R. Holderegger, and C. Brochmann. 2010. Evolutionary consequences of autopolyploidy. *New Phytologist* 186: 5-17.

Petit, C., F. Bretagnolle, and F. Felber. 1999. Evolutionary consequences of diploid-polyploid hybrid zones in wild species. *Trends in Ecology & Evolution* 14: 306-311.

Petit, C., P. Lesbros, X. J. Ge, and J. D. Thompson. 1997. Variation in flowering phenology and selfing rate across a contact zone between diploid and tetraploid *Arrhenatherum elatius* (Poaceae). *Heredity* 79: 31-40.

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Wien, Austria.

Ramsey, J. 2011. Polyploidy and ecological adaptation in wild yarrow. *Proceedings of the National Academy of Sciences, USA* 108: 7096-7101.

Ramsey, J., and D. W. Schemske. 2002. Neopolyploidy in flowering plants. *Annual Review of Ecology and Systematics* 33: 589-639.

Rausch, J. H., and M. T. Morgan. 2005. The effect of self-fertilization, inbreeding depression, and population size on autopolyploid establishment. *Evolution* 59: 1867-1875.

Rešetnik, I., B. Frajman, S. Bogdanović, F. Ehrendorfer, and P. Schönswetter. 2014. Disentangling relationships among the diploid members of the intricate genus *Knautia* (Caprifoliaceae, Dipsacoideae). *Molecular Phylogenetics and Evolution* 74: 97-110.

Ripley, B. D. 1977. Modeling spatial patterns. *Journal of the Royal Statistical Society, B, Methodological* 39: 172-212.

Rodríguez, D. J. 1996. A model for the establishment of polyploidy in plants. *American Naturalist* 147: 33-46.

Rothera, S. L., and A. J. Davy. 1986. Polyploidy and habitat differentiation in *Deschampsia cespitosa*. *New Phytologist* 102: 449-467.

Šafářová, L., and M. Duchoslav. 2010. Cytotype distribution in mixed populations of polyploid *Allium oleraceum* measured at a microgeographic scale. *Preslia* 82: 107-126.

Schönswetter, P., J. Suda, M. Popp, H. Weiss-Schneeweiss, and C. Brochmann. 2007a. Circumpolar phylogeography of *Juncus biglumis* (Juncaceae) inferred from AFLP fingerprints, cpDNA sequences, nuclear DNA content and chromosome numbers. *Molecular Phylogenetics and Evolution* 42: 92-103.

Schönswetter, P., M. Lachmayer, C. Lettner, D. Prehsler, S. Rechnitzer, D. S. Reich, M. Sonnleitner, et al. 2007b. Sympatric diploid and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps are separated along an altitudinal gradient. *Journal of Plant Research* 120: 721-725.

Segraves, K. A., and J. N. Thompson. 1999. Plant polyploidy and pollination: floral traits and insect visits to diploid and tetraploid *Heuchera grossulariifolia*. *Evolution* 53: 1114-1127.

Šingliarová, B., J. Chrtek, I. Plačková, and P. Mráz. 2011. Allozyme variation in diploid, polyploid and mixed-ploidy populations of the *Pilosella alpicola* Group (Asteraceae): relation to morphology, origin of polyploids and breeding system. *Folia Geobotanica* 46: 387-410.

Soltis, D. E., and P. S. Soltis. 1999. Polyploidy: recurrent formation and genome evolution. *Trends in Ecology & Evolution* 14: 348-352.

Soltis, D. E., V. A. Albert, J. Leebens-Mack, C. D. Bell, A. H. Paterson, C. F. Zheng, D. Sankoff, et al. 2009. Polyploidy and angiosperm diversification. *American Journal of Botany* 96: 336-348.

Sonnleitner, M., R. Flatscher, P. E. García, J. Rauchová, J. Suda, G. M. Schneeweiss, K. Hülber, et al. 2010. Distribution and habitat segregation on different spatial scales among diploid, tetraploid and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps. *Annals of Botany* 106: 967-977.

StatSoft. 2007. STATISTICA (data analysis software system), version 8.0. StatSoft Inc., Tulsa, Oklahoma, USA.

Štěpánek, J. 1982. Die Chromosomenzahlen von tschechoslowakischen Arten der Gattung *Knautia* L. (Dipsacaceae). *Folia Geobotanica et Phytotaxonomica* 17: 359-386.

Štěpánek, J. 1989. Chrastavec rolní krkonošský – *Knautia arvensis* (L.) Coulter subsp. *pseudolongifolia* (Szabó) O. Schwarz. *In* B. Slavík [ed.], Studie ČSAV. 10. Vybrané ohrožené druhy flóry ČSR, 25-36. Academia, Prague, Czech Republic.

Štěpánek, J. 1997. *Knautia* L. – chrastavec. *In* B. Slavík [ed.], Květena České republiky, vol. 6, 543-554. Academia, Praha, Czech Republic.

Suda, J., and T. Herben. 2013. Ploidy frequencies in plants with ploidy heterogeneity: fitting a general gametic model to empirical population data. *Proceedings of the Royal Society, B, Biological Sciences* 280: 20122387.

Suda, J., A. Krahulcová, P. Trávníček, and F. Krahulec. 2006. Ploidy level versus DNA ploidy level: an appeal for consistent terminology. *Taxon* 55: 447-450.

te Beest, M., J. J. Le Roux, D. M. Richardson, A. K. Brysting, J. Suda, M. Kubešová, and P. Pyšek. 2012. The more the better? The role of polyploidy in facilitating plant invasions. *Annals of Botany* 109: 19-45.

ter Braak, C. J. F., and P. Šmilauer. 2002. CANOCO Reference Manual and CanoDraw for Windows User´s Guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power, Ithaca, New York, USA.

Thompson, S. L., and J. Whitton. 2006. Patterns of recurrent evolution and geographic parthenogenesis within apomictic polyploid Easter daises (*Townsendia hookeri*). *Molecular Ecology* 15: 3389-3400.

Tichý, L. 2002. JUICE, software for vegetation classification. *Journal of Vegetation Science* 13: 451-453.

Trávníček, P., Z. Dočkalová, R. Rosenbaumová, B. Kubátová, Z. Szelag, and J. Chrtek. 2011a. Bridging global and microregional scales: ploidy distribution in *Pilosella echioides* (Asteraceae) in central Europe. *Annals of Botany* 107: 443-454.

Trávníček, P., B. Kubátová, V. Čurn, J. Rauchová, E. Krajníková, J. Jersáková, and J. Suda. 2011b. Remarkable coexistence of multiple cytotypes of the *Gymnadenia conopsea* aggregate (the fragrant orchid): evidence from flow cytometry. *Annals of Botany* 107: 77-87.

Trávníček, P., J. Jersáková, B. Kubátová, J. Krejčíková, R. M. Bateman, M. Lučanová, E. Krajníková, et al. 2012. Minority cytotypes in European populations of the *Gymnadenia conopsea* complex (Orchidaceae) greatly increase intraspecific and intrapopulation diversity. *Annals of Botany* 110: 977-986.

van Dijk, P. J., M. V. Hartog, and W. Van Delden. 1992. Single cytotype areas in autopolyploid *Plantago media* L. *Biological Journal of the Linnean Society* 46: 315-331.

Vange, V. 2002. Breeding system and inbreeding depression in the clonal plant species *Knautia arvensis* (Dipsacaceae): implications for survival in abandoned grassland. *Biological Conservation* 108: 59-67.

Weiss-Schneeweiss, H., K. Emadzade, T. -S. Jang, and G. M. Schneeweiss. 2013. Evolutionary consequences, constraints and potential of polyploidy in plants. *Cytogenetic and Genome Research* 140: 137-150.

Wood, T. E., N. Takebayashi, M. S. Barker, I. Mayrose, P. B. Greenspoon, and L. H. Rieseberg. 2009. The frequency of polyploid speciation in vascular plants. *Proceedings of the National Academy of Sciences, USA* 106: 13875-13879.

Appendix S1. List of *Knautia arvensis* subpopulations from a diploid-tetraploid primary contact zone in a relict serpentine site in the Slavkovský les Mts., western Bohemia, the Czech Republic.

Subpopulation no.	Approximate number of plants	No. of plants subjected to flow cytometric analysis	DNA ploidy level(s)	Proportion of 4x plants [%]	Plots	Vegetation sample	Latitude	Longitude
1	10-100	9	2, 4	30			50° 02' 15.3"N	12° 45' 58.3"E
2	> 500	68	2	0		no. 1	50° 02' 15.9"N	12° 45' 59.3"E
3	10-100	6	4	100		no. 7	50° 02' 16.7"N	12° 46' 01.0"E
4	250-500	37	4	100			50° 02' 18.3"N	12° 46' 00.2"E
5	10-100	3	4	100			50° 02' 19.6"N	12° 45' 59.5"E
6	100-250	8	4	100			50° 02' 17.4"N	12° 45' 47.0"E
7	10-100	3	2, 4	70			50° 02' 17.0"N	12° 45' 45.4"E
8	100-250	10	4	100			50° 02' 13.9"N	12° 45' 46.7"E
9	10-100	2	4	100			50° 02' 20.5"N	12° 46' 02.7"E
10	< 10	2	4	100			50° 02' 11.7"N	12° 45' 48.8"E
11	10-100	5	4	100			50° 02' 09.9"N	12° 45' 48.2"E
12	> 500	26	4	100		no. 5	50° 02' 11.7"N	12° 45' 46.5"E
13	250-500	29	4	100			50° 02' 12.5"N	12° 45' 44.5"E
14	10-100	4	4	100			50° 02' 10.8"N	12° 45' 47.4"E
15	> 500	33	4	100		no. 6	50° 02' 16.0"N	12° 45' 50.0"E
16	10-100	6	2	0		no. 2	50° 02' 14.4"N	12° 45' 54.5"E
17	10-100	4	2	0			50° 02' 16.7"N	12° 45' 54.3"E
18	10-100	8	2	0		no. 8	50° 02' 16.4"N	12° 45' 54.5"E
19	10-100	9	4	100		no. 9	50° 02' 18.0"N	12° 45' 53.6"E
20	10-100	8	4	100			50° 02' 18.2"N	12° 45' 52.8"E
21	> 500	18	2, 4	70	M1	fine-scale v.	50° 02' 17.5"N	12° 45' 57.4"E
22	10-100	7	2	0			50° 02' 16.9"N	12° 45' 57.5"E
23	10-100	7	4	100			50° 02' 14.1"N	12° 46' 03.0"E
24	10-100	3	4	100			50° 02' 14.8"N	12° 45' 57.7"E
25	< 10	3	4	100			50° 02' 11.1"N	12° 46' 05.7"E
26	10-100	7	4	100			50° 03' 22.1"N	12° 47' 16.1"E
27	100-250	14	4	100			50° 03' 09.8"N	12° 47' 07.4"E
28	100-250	8	4	100			50° 02' 57.1"N	12° 46' 41.8"E
29	100-250	17	2	0			50° 02' 19.2"N	12° 45' 42.5"E
30	10-100	9	4	100			50° 02' 21.0"N	12° 45' 23.6"E

31	100-250	12	4	100			50° 02' 18.7"N	12° 45' 02.5"E
32	250-500	104	2, 4	30	M3		50° 02' 14.5"N	12° 45' 43.2"E
33	250-500	97	2	0			50° 02' 17.6"N	12° 45' 42.4"E
34	100-250	12	2	0		no. 3	50° 02' 17.7"N	12° 45' 40.4"E
35	10-100	6	2	0		no. 10	50° 02' 19.3"N	12° 45' 43.8"E
36	100-250	12	4	100		no. 12	50° 02' 24.0"N	12° 45' 49.6"E
37	250-500	17	4	100			50° 02' 34.3"N	12° 45' 54.4"E
38	100-250	8	4	100			50° 02' 40.8"N	12° 45' 58.4"E
39	100-250	13	4	100			50° 02' 49.2"N	12° 46' 07.4"E
40	10-100	3	4	100			50° 02' 59.8"N	12° 46' 38.8"E
41	10-100	3	4	100			50° 03' 09.6"N	12° 46' 49.2"E
42	250-500	15	4	100			50° 03' 10.8"N	12° 46' 53.8"E
43	250-500	12	4	100			50° 03' 12.1"N	12° 46' 56.5"E
44	100-250	11	4	100			50° 03' 13.8"N	12° 46' 56.4"E
45	250-500	16	4	100			50° 03' 16.5"N	12° 47' 01.3"E
46	10-100	6	4	100			50° 03' 17.4"N	12° 47' 04.0"E
47	250-500	16	4	100			50° 03' 21.2"N	12° 47' 10.0"E
48	100-250	8	4	100			50° 03' 21.6"N	12° 47' 08.5"E
49	10-100	11	4	100			50° 03' 19.4"N	12° 47' 01.8"E
50	100-250	10	4	100			50° 03' 14.7"N	12° 46' 54.0"E
51	> 500	17	4	100			50° 03' 14.4"N	12° 46' 49.7"E
52	250-500	16	4	100			50° 03' 11.7"N	12° 46' 47.3"E
53	250-500	11	4	100			50° 03' 01.8"N	12° 46' 37.2"E
54	100-250	8	2	0			50° 02' 19.8"N	12° 45' 42.8"E
55	100-250	9	2	0		no. 11	50° 02' 21.6"N	12° 45' 45.7"E
56	10-100	5	2	0			50° 02' 18.2"N	12° 45' 37.7"E
57	10-100	4	4	100		no. 4	50° 02' 12.2"N	12° 45' 37.2"E
58	250-500	18	4	100			50° 02' 11.0"N	12° 45' 37.9"E
59	10-100	8	4	100			50° 02' 15.1"N	12° 45' 24.2"E
60	> 500	21	4	100			50° 02' 14.4"N	12° 45' 21.3"E
61	100-250	14	4	100		no. 35	50° 02' 11.7"N	12° 45' 14.0"E
62	100-250	9	4	100		no. 36	50° 02' 13.3"N	12° 45' 05.8"E
63	100-250	12	4	100		no. 13	50° 02' 14.2"N	12° 44' 58.1"E
64	10-100	4	4	100			50° 02' 14.3"N	12° 44' 20.8"E
65	10-100	4	4	100			50° 01' 56.3"N	12° 43' 47.0"E
66	10-100	2	4	100			50° 01' 54.6"N	12° 43' 31.8"E

67	< 10	1	4	100			50° 01' 51.1"N	12° 43' 31.8"E
68	100-250	5	4	100			50° 01' 50.2"N	12° 43' 28.0"E
69	< 10	2	4	100			50° 01' 39.0"N	12° 43' 24.1"E
70	< 10	2	4	100			50° 01' 37.0"N	12° 43' 26.9"E
71	10-100	3	4	100			50° 01' 37.9"N	12° 43' 28.8"E
72	10-100	3	4	100			50° 01' 44.5"N	12° 43' 35.0"E
73	250-500	7	4	100			50° 01' 45.4"N	12° 43' 35.5"E
74	> 500	33	4	100			50° 01' 48.2"N	12° 43' 37.7"E
75	10-100	3	4	100			50° 01' 49.6"N	12° 43' 38.3"E
76	< 10	1	4	100			50° 01' 47.1"N	12° 43' 45.2"E
77	10-100	4	4	100			50° 01' 44.8"N	12° 43' 49.6"E
78	10-100	5	4	100			50° 01' 45.2"N	12° 43' 59.0"E
79	> 500	16	4	100			50° 01' 43.5"N	12° 44' 01.3"E
80	100-250	12	4	100			50° 01' 45.1"N	12° 44' 04.8"E
81	100-250	11	4	100		no. 40	50° 01' 48.4"N	12° 44' 10.2"E
82	100-250	10	4	100		no. 24	50° 01' 50.5"N	12° 44' 16.4"E
83	10-100	5	2, 4	80			50° 01' 52.4"N	12° 44' 20.3"E
84	> 500	5	2	0		no. 16	50° 01' 55.9"N	12° 44' 17.0"E
85	100-250	7	4	100			50° 01' 58.6"N	12° 44' 14.4"E
86	10-100	7	4	100			50° 01' 59.8"N	12° 44' 11.0"E
87	250-500	7	4	100			50° 02' 00.8"N	12° 44' 06.6"E
88	100-250	11	4	100			50° 02' 01.1"N	12° 44' 01.6"E
89	250-500	24	4	100		no. 17	50° 01' 58.0"N	12° 44' 13.9"E
90	10-100	4	4	100			50° 01' 53.4"N	12° 44' 12.1"E
91	100-250	8	4	100			50° 01' 52.8"N	12° 44' 15.8"E
92	< 10	1	2	0			50° 01' 52.8"N	12° 44' 23.5"E
93	10-100	8	2	0		no. 18	50° 01' 53.9"N	12° 44' 27.9"E
94	100-250	8	4	100		no. 19	50° 01' 58.4"N	12° 44' 27.5"E
95	250-500	93	2, 4	90	M4		50° 01' 59.4"N	12° 44' 25.4"E
96	10-100	11	2	0		no. 20	50° 01' 57.8"N	12° 44' 20.8"E
97	10-100	9	4	100			50° 01' 55.5"N	12° 44' 14.2"E
98	10-100	15	2	0			50° 01' 57.0"N	12° 44' 16.9"E
99	10-100	6	4	100			50° 01' 56.1"N	12° 44' 09.7"E
100	10-100	10	2	0		no. 14	50° 02' 12.5"N	12° 44' 50.0"E
101	10-100	8	4	100			50° 02' 07.4"N	12° 44' 36.3"E
102	100-250	13	4	100			50° 02' 05.9"N	12° 44' 32.1"E

103	100-250	12	4	100			50° 02' 00.9"N	12° 44' 33.7"E
104	10-100	11	2	0			50° 01' 56.2"N	12° 44' 25.0"E
105	100-250	21	2	0		no. 15	50° 01' 55.1"N	12° 44' 25.5"E
106	250-500	80	2, 4, 6	40	M2	fine-scale v.	50° 01' 57.3"N	12° 44' 14.8"E
107	10-100	4	4	100			50° 01' 59.8"N	12° 44' 16.3"E
108	10-100	6	4	100		no. 23	50° 02' 01.1"N	12° 44' 21.8"E
109	10-100	8	4	100			50° 02' 02.9"N	12° 44' 21.0"E
110	100-250	16	4	100			50° 02' 05.7"N	12° 44' 22.2"E
111	250-500	31	4	100			50° 02' 09.3"N	12° 45' 23.6"E
112	10-100	9	4	100			50° 02' 07.0"N	12° 45' 13.9"E
113	10-100	11	4	100			50° 02' 03.5"N	12° 45' 09.7"E
114	100-250	12	4	100			50° 02' 15.1"N	12° 45' 29.0"E
115	100-250	17	2, 4	70			50° 02' 13.8"N	12° 44' 43.5"E
116	250-500	34	4	100		no. 22	50° 02' 13.6"N	12° 44' 40.3"E
117	100-250	13	4	100			50° 02' 12.2"N	12° 44' 40.7"E
118	< 10	5	4	100			50° 02' 09.5"N	12° 44' 40.8"E
119	100-250	14	4	100		no. 38	50° 02' 07.8"N	12° 44' 43.3"E
120	10-100	3	4	100			50° 02' 07.5"N	12° 44' 45.9"E
121	10-100	8	4	100			50° 02' 22.0"N	12° 45' 22.1"E
122	< 10	1	4	100			50° 02' 25.2"N	12° 45' 08.0"E
123	10-100	7	4	100			50° 02' 21.7"N	12° 44' 40.2"E
124	> 500	26	4	100			50° 02' 19.3"N	12° 44' 40.4"E
125	250-500	12	4	100			50° 02' 07.0"N	12° 45' 12.9"E
126	100-250	10	4	100			50° 02' 23.3"N	12° 44' 18.6"E
127	10-100	4	4	100			50° 02' 19.5"N	12° 44' 24.7"E
128	100-250	16	4	100			50° 02' 17.9"N	12° 44' 35.5"E
129	100-250	11	4	100			50° 02' 08.1"N	12° 44' 29.9"E
130	100-250	13	4	100			50° 02' 11.5"N	12° 44' 13.1"E
131	100-250	13	4	100			50° 02' 12.1"N	12° 44' 12.5"E
132	100-250	21	4	100			50° 02' 14.8"N	12° 44' 09.5"E
133	10-100	7	4	100			50° 02' 17.4"N	12° 44' 09.1"E
134	100-250	7	4	100			50° 02' 53.4"N	12° 46' 04.2"E
135	100-250	14	4	100			50° 02' 33.2"N	12° 45' 21.3"E
136	10-100	7	4	100			50° 02' 33.9"N	12° 45' 23.7"E
137	< 10	2	2	0			50° 02' 36.2"N	12° 45' 27.1"E
138	100-250	12	4	100			50° 02' 26.0"N	12° 45' 23.3"E

139	10-100	6	4	100	no. 29	50° 02' 21.3"N	12° 45' 25.6"E
140	10-100	8	4	100		50° 02' 24.0"N	12° 45' 24.0"E
141	100-250	17	4	100		50° 02' 25.1"N	12° 45' 54.0"E
142	10-100	13	4	100		50° 02' 25.8"N	12° 45' 58.1"E
143	250-500	29	4	100		50° 02' 25.4"N	12° 46' 01.3"E
144	10-100	10	4	100		50° 02' 24.3"N	12° 46' 03.9"E
145	100-250	25	4	100		50° 02' 21.3"N	12° 46' 03.6"E
146	10-100	16	4	100		50° 02' 08.0"N	12° 45' 54.2"E
147	10-100	4	4	100		50° 02' 08.1"N	12° 45' 52.4"E
148	250-500	23	4	100		50° 02' 08.0"N	12° 45' 44.5"E
149	10-100	18	4	100		50° 02' 10.7"N	12° 45' 34.4"E
150	100-250	13	4	100	no. 28	50° 02' 12.6"N	12° 45' 29.0"E
151	100-250	45	4	100		50° 02' 12.6"N	12° 45' 24.0"E
152	250-500	24	4	100		50° 02' 03.8"N	12° 45' 14.9"E
153	10-100	6	4	100		50° 02' 01.4"N	12° 45' 12.5"E
154	100-250	35	2, 4	90		50° 02' 00.2"N	12° 45' 10.4"E
155	100-250	13	4	100		50° 01' 59.7"N	12° 45' 07.9"E
156	10-100	13	2, 4	20		50° 02' 06.5"N	12° 44' 49.6"E
157	10-100	7	4	100		50° 02' 04.6"N	12° 44' 37.0"E
158	10-100	6	4	100	no. 21	50° 01' 53.3"N	12° 44' 13.4"E
159	10-100	10	4	100		50° 01' 51.5"N	12° 44' 12.9"E
160	10-100	12	4	100		50° 01' 48.5"N	12° 44' 11.6"E
161	10-100	8	4	100		50° 01' 46.6"N	12° 44' 08.7"E
162	10-100	16	4	100		50° 01' 44.9"N	12° 44' 05.5"E
163	10-100	15	4	100		50° 01' 40.5"N	12° 43' 54.3"E
164	< 10	5	4	100		50° 02' 18.3"N	12° 45' 11.0"E
165	100-250	27	4	100		50° 02' 10.4"N	12° 44' 16.0"E
166	10-100	12	4	100		50° 02' 04.2"N	12° 44' 08.6"E
167	< 10	4	4	100		50° 02' 00.2"N	12° 43' 54.2"E
168	> 500	40	4	100		50° 01' 53.2"N	12° 43' 48.7"E
169	100-250	19	4	100		50° 01' 52.8"N	12° 43' 44.6"E
170	250-500	24	4	100		50° 02' 16.2"N	12° 45' 19.8"E
171	10-100	4	4	100		50° 02' 15.2"N	12° 45' 11.2"E
172	100-250	22	4	100		50° 02' 16.0"N	12° 45' 07.4"E
173	10-100	11	4	100		50° 02' 10.4"N	12° 44' 44.3"E
174	10-100	3	4	100		50° 02' 07.6"N	12° 44' 38.9"E

175	10-100	12	4	100		50° 02' 02.2"N	12° 45' 05.4"E
176	< 10	2	4	100		50° 02' 04.7"N	12° 45' 01.1"E
177	< 10	3	2	0		50° 02' 08.7"N	12° 44' 49.4"E
178	< 10	2	2	0		50° 02' 09.7"N	12° 44' 50.4"E
179	< 10	4	2	0		50° 02' 10.4"N	12° 44' 48.6"E
180	< 10	5	4	100		50° 02' 10.3"N	12° 44' 41.8"E
181	10-100	10	4	100		50° 01' 57.7"N	12° 44' 03.0"E
182	250-500	41	4	100		50° 01' 59.2"N	12° 44' 02.7"E
183	< 10	1	4	100		50° 01' 55.9"N	12° 45' 18.2"E
184	> 500	23	4	100		50° 01' 57.2"N	12° 45' 14.4"E
185	> 500	23	4	100		50° 01' 58.7"N	12° 45' 12.9"E
186	250-500	28	4	100		50° 02' 02.7"N	12° 45' 06.9"E
187	> 500	24	4	100	no. 26	50° 02' 05.4"N	12° 45' 03.9"E
188	100-250	15	2	100	no. 33	50° 02' 09.1"N	12° 44' 59.8"E
189	250-500	20	2, 3	0	no. 27	50° 02' 15.8"N	12° 44' 49.8"E
190	> 500	11	4	100		50° 02' 18.6"N	12° 44' 41.7"E
191	> 500	20	4	100		50° 02' 22.3"N	12° 44' 31.0"E
192	250-500	12	4	100		50° 02' 25.0"N	12° 44' 25.0"E
193	10-100	6	4	100		50° 02' 24.3"N	12° 44' 13.2"E
194	< 10	2	4	100		50° 02' 26.7"N	12° 44' 16.3"E
195	100-250	13	4	100		50° 02' 18.7"N	12° 44' 13.9"E
196	< 10	3	4	100		50° 02' 16.7"N	12° 44' 17.4"E
197	< 10	2	4	100		50° 02' 10.9"N	12° 44' 23.5"E
198	100-250	19	4	100		50° 02' 09.2"N	12° 44' 23.7"E
199	100-250	16	4	100		50° 02' 07.5"N	12° 44' 25.7"E
200	10-100	12	4	100		50° 02' 04.4"N	12° 44' 26.8"E
201	10-100	11	2	0		50° 01' 53.1"N	12° 44' 31.4"E
202	100-250	11	2	0		50° 01' 49.4"N	12° 44' 26.1"E
203	100-250	19	2	0	no. 25	50° 01' 47.8"N	12° 44' 24.3"E
204	10-100	8	4	100		50° 01' 45.5"N	12° 44' 15.2"E
205	< 10	2	4	100		50° 01' 49.5"N	12° 44' 04.1"E
206	< 10	4	4	100		50° 01' 53.6"N	12° 43' 55.7"E
207	< 10	4	4	100		50° 01' 49.2"N	12° 43' 54.8"E
208	< 10	1	4	100		50° 02' 01.7"N	12° 45' 13.7"E
209	100-250	13	4	100		50° 02' 03.8"N	12° 45' 11.9"E
210	10-100	6	4	100		50° 02' 05.0"N	12° 45' 10.9"E

211	10-100	7	4	100		50° 02' 08.5"N	12° 45' 04.3"E
212	250-500	14	2	0		50° 02' 11.2"N	12° 44' 59.8"E
213	10-100	3	2	0	no. 32	50° 02' 13.0"N	12° 44' 56.8"E
214	100-250	8	2	0		50° 02' 15.6"N	12° 44' 53.7"E
215	10-100	13	2	0		50° 01' 59.2"N	12° 44' 23.7"E
216	250-500	29	2	0		50° 01' 56.8"N	12° 44' 23.8"E
217	< 10	2	2	0		50° 01' 48.7"N	12° 44' 17.5"E
218	100-250	15	2	0	no. 39	50° 01' 47.1"N	12° 44' 18.7"E
219	10-100	7	2	0		50° 01' 47.1"N	12° 44' 21.9"E
220	< 10	2	2	0		50° 01' 45.8"N	12° 44' 24.9"E
221	100-250	13	4	100		50° 01' 59.3"N	12° 44' 37.4"E
222	< 10	1	4	100		50° 02' 01.2"N	12° 44' 49.2"E
223	< 10	2	4	100		50° 01' 58.9"N	12° 44' 53.0"E
224	10-100	11	4	100		50° 02' 19.0"N	12° 44' 55.9"E
225	< 10	2	4	100		50° 02' 32.0"N	12° 45' 18.7"E
226	10-100	13	4	100		50° 02' 21.9"N	12° 45' 01.9"E
227	> 500	13	4	100		50° 02' 19.6"N	12° 45' 00.1"E
228	< 10	3	4	100		50° 02' 11.7"N	12° 45' 35.6"E
229	10-100	11	2	0	no. 30	50° 02' 19.7"N	12° 45' 28.6"E
230	10-100	7	2	0	no. 31	50° 02' 22.3"N	12° 45' 34.0"E
231	< 10	3	4	100		50° 02' 26.5"N	12° 45' 32.1"E
232	< 10	3	4	100		50° 02' 28.1"N	12° 45' 45.3"E
233	250-500	17	4	100		50° 02' 27.6"N	12° 45' 48.3"E
234	10-100	12	4	100		50° 02' 28.6"N	12° 45' 51.0"E
235	10-100	4	4	100		50° 02' 30.0"N	12° 45' 57.1"E
236	< 10	5	4	100		50° 02' 28.7"N	12° 46' 00.7"E
237	< 10	3	4	100		50° 02' 27.9"N	12° 46' 05.2"E
238	10-100	4	4	100		50° 02' 27.8"N	12° 46' 07.2"E
239	100-250	11	4	100		50° 02' 07.9"N	12° 44' 08.8"E
240	10-100	5	4	100		50° 02' 09.8"N	12° 44' 07.4"E
241	10-100	7	4	100		50° 02' 10.4"N	12° 44' 05.1"E
242	10-100	5	4	100		50° 02' 12.2"N	12° 44' 03.0"E
243	10-100	9	2, 4	60		50° 02' 14.8"N	12° 44' 00.1"E
244	10-100	5	4	100		50° 02' 13.4"N	12° 43' 56.6"E
245	10-100	11	4	100		50° 02' 09.2"N	12° 43' 56.3"E
246	100-250	11	4	100		50° 02' 09.4"N	12° 43' 52.5"E

247	10-100	7	4	100		50° 02' 07.8"N	12° 43' 47.2"E
248	10-100	8	4	100		50° 02' 06.1"N	12° 43' 41.9"E
249	10-100	9	4	100		50° 02' 01.8"N	12° 43' 39.9"E
250	10-100	7	4	100		50° 02' 00.2"N	12° 43' 36.1"E
251	10-100	8	4	100		50° 01' 59.8"N	12° 43' 39.4"E
252	10-100	9	4	100		50° 01' 49.5"N	12° 43' 34.2"E
253	< 10	4	4	100		50° 01' 38.2"N	12° 43' 36.4"E
254	10-100	5	4	100		50° 01' 37.1"N	12° 43' 39.9"E
255	10-100	9	4	100		50° 01' 39.1"N	12° 43' 42.7"E
256	10-100	10	4	100		50° 01' 40.1"N	12° 43' 44.3"E
257	10-100	5	4	100		50° 01' 45.6"N	12° 43' 49.1"E
258	10-100	5	4	100		50° 01' 48.7"N	12° 43' 50.1"E
259	< 10	3	4	100		50° 01' 53.5"N	12° 44' 09.0"E
260	100-250	7	2	0		50° 01' 55.6"N	12° 44' 23.9"E
261	10-100	6	4	100		50° 02' 00.4"N	12° 44' 40.5"E
262	10-100	12	4	100		50° 02' 01.7"N	12° 44' 43.1"E
263	10-100	13	2	0	no. 37	50° 02' 05.9"N	12° 44' 47.5"E
264	10-100	10	2	0		50° 02' 11.2"N	12° 45' 02.8"E
265	10-100	7	2	0	no. 34	50° 02' 10.7"N	12° 45' 07.3"E
266	10-100	8	4	100		50° 02' 12.9"N	12° 45' 11.7"E
267	< 10	5	4	100		50° 02' 14.9"N	12° 45' 11.9"E
268	< 10	4	4	100		50° 02' 18.4"N	12° 45' 15.1"E
269	10-100	9	4	100		50° 02' 30.0"N	12° 45' 38.9"E
270	< 10	4	2	0		50° 02' 22.4"N	12° 45' 45.9"E
271	< 10	4	4	100		50° 02' 22.7"N	12° 45' 58.3"E
272	10-100	6	4	100		50° 02' 20.8"N	12° 46' 06.3"E
273	10-100	15	4	100		50° 02' 19.4"N	12° 46' 07.9"E
274	10-100	7	4	100		50° 02' 19.0"N	12° 46' 11.7"E
275	10-100	8	4	100		50° 01' 59.9"N	12° 44' 20.1"E
276	10-100	5	4	100		50° 02' 03.5"N	12° 44' 17.7"E
277	250-500	7	4	100		50° 02' 06.8"N	12° 44' 16.9"E
278	250-500	13	4	100		50° 02' 10.0"N	12° 44' 19.0"E
279	10-100	5	4	100		50° 02' 13.6"N	12° 44' 19.8"E
280	10-100	4	4	100		50° 02' 26.6"N	12° 46' 04.6"E
281	10-100	9	4	100		50° 02' 30.9"N	12° 46' 03.5"E
282	10-100	6	4	100		50° 02' 55.8"N	12° 46' 29.0"E

283	10-100	5	4	100	50° 02' 59.6"N	12° 46' 25.8"E
284	10-100	11	4	100	50° 03' 04.4"N	12° 46' 31.4"E
285	10-100	7	4	100	50° 03' 07.6"N	12° 46' 32.8"E
286	100-250	10	4	100	50° 03' 10.6"N	12° 46' 34.4"E
287	100-250	10	4	100	50° 03' 14.1"N	12° 46' 39.9"E
288	10-100	6	4	100	50° 03' 15.6"N	12° 46' 44.6"E
289	10-100	5	4	100	50° 03' 18.5"N	12° 46' 47.1"E
290	10-100	7	4	100	50° 03' 20.7"N	12° 46' 49.6"E
291	10-100	10	4	100	50° 03' 23.8"N	12° 46' 55.5"E
292	250-500	11	4	100	50° 03' 25.2"N	12° 46' 57.5"E
293	100-250	12	4	100	50° 03' 27.1"N	12° 46' 59.4"E
294	250-500	13	4	100	50° 03' 24.9"N	12° 47' 03.1"E
295	10-100	6	4	100	50° 03' 21.7"N	12° 46' 57.9"E
296	10-100	12	4	100	50° 03' 20.2"N	12° 46' 54.9"E
297	10-100	9	4	100	50° 03' 17.7"N	12° 46' 50.2"E
298	10-100	4	4	100	50° 03' 15.2"N	12° 46' 54.0"E
299	100-250	10	4	100	50° 03' 13.1"N	12° 46' 55.3"E
300	10-100	5	4	100	50° 03' 13.8"N	12° 46' 59.6"E
301	10-100	6	4	100	50° 03' 09.3"N	12° 47' 02.4"E

Appendix S2. Vegetation samples characterizing environmental conditions in 20 uniformly diploid and 20 uniformly tetraploid subpopulations of Knautia arvensis. (A) Presence and relative coverage of particular plant species in vegetation samples, separately for the tree main vegetation layers. (B) Values of additional environmental variables inferred either directly in situ or derived from species composition data.

A

Layer	Species	Relative coverage of species in particular vegetation samples [%]																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
moss	*Ceratodon purpureus*	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
moss	*Cetraria islandica*	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
moss	*Cladonia* sp.	0.5	0	0	0.5	0	0	0	1	1	0	0	1	0	0	0.5	0	0	0.5	0	0.5
moss	*Dicranella heteromala*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
moss	*Dicranum polysetum*	0.5	25	0.5	1	4	0.1	1	0.5	0.5	0	0	0.5	0.5	0.5	3	0.5	0	1	2	35
moss	*Hylocomium splendens*	0	0	0	0	0	0.5	0	40	40	5	1	0	0	40	0	0	10	0	3	5
moss	*Hypnum cupressiforme*	0.5	15	0	0.1	1	0	5	0	0	1	0	0	0	0	0.5	0	0	0	0	0
moss	*Lepidozia reptans*	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0.5	4	0
moss	*Leucobryum glaucum*	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	1	0	0	0	0.5	0.5
moss	*Plagiomnium cuspidatum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
moss	*Pleurozium schreberi*	93	25	15	25	20	10	5	1	1	0	0	35	5	20	15	15	25	25	5	40
moss	*Polytrichum formosum*	0.5	0.5	0.5	0.5	1	0	0	2	2	0.5	1	1	0	3	0.5	0	5	0	0.5	1
moss	*Rhytidiadelphus squarrosus*	0.5	0	40	25	27	75	35	50	50	40	50	5	25	3	0.5	23	40	55	25	3
moss	*Scleropodium purum*	0	0	5	25	0	3	5	0	0	30	35	8	40	0	30	2	0	5	55	5
moss	*Sphagnum* sp.	0	0	0	0	0	0	0	0	0	0.5	10	0	0	0	0	0	0	0	0	0
moss	*Thuidium tamariscinum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Agrostis capillaris*	0.5	5	10	15	25	25	60	5	30	10	50	5	25	2	6	10	15	20	10	9
herb	*Achillea millefolium*	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Anthoxanthum odoratum*	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Asplenium adulterinum*	0	0.5	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Asplenium cuneifolium*	0.1	0.5	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Avenella flexuosa*	1	0.5	6	10	15	15	10	0.1	2	3	3	1	10	3	0.5	0.5	2	15	8	7
herb	*Bistorta major*	0	0	0	0	0	0	0	0	0	0	0.1	0	0.1	0	0	0	0	0.5	0	0
herb	*Calamagrostis arundinacea*	0	0.1	0	0	0	0	0	0	1	1	0	0.1	0	0	0	0	10	0	0	0
herb	*Calamagrostis epigejos*	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0
herb	*Calluna vulgaris*	0	0	3.5	0	1	0	0	0	0	0	0	0	0	1	0	0.5	1	0	0	0.1

herb	*Campanula rotundifolia*	0	1	2	1	0.1	0	0	0.1	0.5	0	0	0	0	0	0	0	0	0	4	0
herb	*Cardamine pratensis*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0
herb	*Carex montana*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	0	1	5
herb	*Carex nigra*	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Cerastium alsinifolium*	0	0	0.1	0	0	0	0	0	0.5	0	0	0	0	0	0	1	0.5	0.5	0	0
herb	*Cirsium palustre*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Dactylis glomerata*	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Danthonia decumbens*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Deschampsia cespitosa*	0	0	5	1	0	0.1	1	1	1	0	3	4	4	2	1	3	15	4	5	5
herb	*Dryopteris dilatata*	0	0	0	0	0	0	0	0	0.1	0	0.5	0.1	0	0	0	0	0	0.1	0	0
herb	*Erica carnea*	0.5	0	0	0	0.1	0	0	0	0	1	0	0	0	5	0.5	0	1	0	0	0
herb	*Festuca ovina*	1	30	10	0.1	15	15	0	8	25	5	5	5	2	1	7	12	15	2	10	3
herb	*Festuca rubra*	0	0	0	0	0	0	0.5	0	0	0	0	0	2	0	0	15	0	0	0	1
herb	*Fragaria vesca*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Galium saxatile*	0.5	0.5	0.1	0.5	0.5	0.1	0	0	3	0.5	1	0.5	0.5	0.5	0	1	0.5	1	0	0.5
herb	*Galium sudeticum*	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Galium verum*	0.5	0.1	1	0.5	0.1	0.5	0	0.5	0	0	0.5	0.5	2	0.5	2	0.5	0.5	0.5	0	0.1
herb	*Hieracium lachenalii*	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Hieracium pilosella*	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Holcus mollis*	0	0	0	0	0	0.1	0	0	0.1	0	0.5	0.1	0	0	0	0	0	0	0	0
herb	*Juncus bulbosus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Larix decidua* (seedling)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Lathyrus pratensis*	0	0	0.1	0	0	0	0.5	0	0	0	0	0	2	0	0	2	0.5	0	0	0.1
herb	*Lathyrus sylvestris*	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Leontodon hispidus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	7	0
herb	*Lotus corniculatus*	0	0	0.5	0	0.1	0.1	0	0.5	0	0	0	0	0	0	0	0	0.1	0	0	0
herb	*Luzula pilosa*	0.5	0	0	0.5	0	1	0.5	0.5	1	2	1	1	0.5	0	0.5	1	1	0.5	2	0.5
herb	*Lychnis flos-cuculi*	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Melampyrum pratense*	6	0.5	0.5	2	0.5	1	0	1	5	5	0.5	5	0.5	2	0	2	0.5	0	0	0
herb	*Molinia caerulea*	0.1	0	35	0	0	0	0	25	0.1	25	5	35	15	30	13	0	1	1	0	0
herb	*Mycelis muralis*	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Narduus stricta*	0	0	0	0	0	1	0	0	0	0	0	0	1	0.1	0	0	0	0	0	0
herb	*Orthilia secunda*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0
herb	*Oxalis acetosella*	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

herb	*Phleum bertolonii*	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Picea abies* (seedling)	0.1	0.5	0.1	0.1	0.1	0.1	0.1	0	0.1	0.5	0.5	0.1	0	0	0	0	0.5	2	0.5	0.5
herb	*Pimpinella major*	0.5	0	0	0	0.5	0.5	10	0.1	0	0	0	5	0	0.1	3	0	0.1	0	2	0.5
herb	*Pinus sylvestris* (seedling)	0.1	0.1	0	0	0	0.1	0.1	0	0.1	0	0	0	0.1	0	0	0	0	0	0	0
herb	*Plantago media*	0	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Poa pratensis*	0	0	0	5	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Polygala chamaebuxus*	0	0	0	0	10	0.1	0	3	0.5	0	0	0	0	2	0.5	0	0.5	2	0.5	0.5
herb	*Polygala vulgaris*	0.5	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Potentilla erecta*	0.5	0	1	0.1	0	0.1	0	0.5	0	0.5	0.5	0.1	2	0.5	1	1	2	0.5	0.5	0
herb	*Prunella vulgaris*	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Pteridium aquilinum*	0	0	0	0	0	0	0	0	0	0	0	0	0	15	7	25	0	5	3	1
herb	*Ranunculus acris*	0	0	0.5	0	0	0	0	0	0	0	0.5	0.1	0.5	0	0	1	2	0.5	0.5	0.5
herb	*Rosa canina* (juvenile)	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Rubus idaeus*	0.5	1	0.1	0.1	0.5	0	0.5	0	0	0	0	0.1	0	0	0	0	0	0	0	0
herb	*Rumex acetosa*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
herb	*Rumex acetosella*	0	0.5	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Senecio ovatus*	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0
herb	*Silene vulgaris*	0.1	3	1	0.5	0.5	1	0.5	4	0	5	2	0.1	3	0	2	0.1	0	0.1	2	0
herb	*Sorbus aucuparia* (juvenile)	0.1	0	0	0.5	0	0.5	0	0.1	0.1	0	0.1	0.5	0.1	0.5	0	0	0	0	0	0
herb	*Stellaria alsine*	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0
herb	*Stellaria graminea*	0	0	0	0	0	0	0.1	0	0.5	0	0.5	0	0	0	0	0	0	0	0	0
herb	*Taraxacum* sect. *Ruderalia*	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Tephroseris crispa*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0
herb	*Thesium alpinum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Trientalis europaea*	0	0	0	0	0	0	0	0	1	0	0	0.5	0	0.5	0	0	0	0	0	0
herb	*Trifolium pratense*	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Trifolium repens*	0.1	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Urtica dioica*	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0
herb	*Vaccinium myrtillus*	70	0	3	4	0	0	0	2	6	5	5	10	20	15	2	2	5	15	4	1
herb	*Vaccinium vitis-idaea*	0	0	0	0	0	0	0	0.5	0.1	0	0.5	0	0.5	0.5	0	0	0.5	0	0.5	0.5
herb	*Veronica chamaedrys*	0.5	0	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Veronica officinalis*	0.5	0	0	0	0.5	0.1	0	0	0.5	0.5	0	0	0	0	0	0	0	0	0	0
herb	*Vicia sepium*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0
herb	*Viola riviniana*	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

tree	*Picea abies*	0	5	10	20	0	5	0	5	0	0	1	0	0	0	10	0	0	7	5	20
tree	*Pinus sylvestris*	0	0	0	20	15	0	50	35	5	40	29	50	40	25	20	0	5	33	0	10
tree	*Larix decidua*	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix S2. (continued)

A

Layer	Species	Relative coverage of species in particular vegetation samples [%]																			
		21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
moss	*Ceratodon purpureus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
moss	*Cetraria islandica*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
moss	*Cladonia* sp.	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0.5	0	0
moss	*Dicranella heteromala*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
moss	*Dicranum polysetum*	1	0	4	0.5	1	15	5	9	1	1	0.5	1	0	1	2	28	10	5	20	25
moss	*Hylocomium splendens*	4	0	0.1	0	0.5	1	0	0	8	0	0	0	0	0	0	0	0	0	0	0
moss	*Hypnum cupressiforme*	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0
moss	*Lepidozia reptans*	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	15	0	0
moss	*Leucobryum glaucum*	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
moss	*Plagiomnium cuspidatum*	0	0	0	0	0	0	0	0	0	0.5	0.5	0	30	0	0	0	0	0	0	0
moss	*Pleurozium schreberi*	10	30	5	3	15	53	50	30	1	70	40	68	0	37	40	35	20	29	20	25
moss	*Polytrichum formosum*	2	0	1	0.5	3	0.5	0	6	0.5	3	0.5	0.5	8	2	5	5	1	1	2	2
moss	*Rhytidiadelphus squarrosus*	2	50	20	20	55	10	0	35	15	0	30	15	50	40	8	0	30	15	2	20
moss	*Scleropodium purum*	10	0.5	65	6	5	0	0	2	0	5	0	1	2	10	30	0	20	20	10	10
moss	*Sphagnum* sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	3
moss	*Thuidium tamariscinum*	0	0	0	0	0	0	0.5	6	0	0	0	0	0	0	10	2	5	0	0	5
herb	*Agrostis capillaris*	5	21	15	35	25	1	2	20	25	10	3	10	30	22	10	15	5	32	5	30
herb	*Achillea millefolium*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Anthoxanthum odoratum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Asplenium adulterinum*	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0.1	0	0
herb	*Asplenium cuneifolium*	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0.5	0	0
herb	*Avenella flexuosa*	0.5	2	5	0	2	15	1	1	0	5	2	5	3	4	2	1	3	3	2	3
herb	*Bistorta major*	0	0	0	0	0	0	0	0	0	0	0.1	0	0.5	0.5	0	0	0	0	0	0
herb	*Calamagrostis arundinacea*	0.1	0	0	0	0	0	0	0	4	0	2	0	0	0	0	0	0	5	0	0

herb	*Calamagrostis epigejos*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Calluna vulgaris*	0	1	0.1	0	0	3	17	0	0	0	0	2	0	0	0	0.5	0.5	2	3	0.5
herb	*Campanula rotundifolia*	0	0.5	0	0.5	0	0.5	0	1	0.1	0	0	0.5	0	0	0.5	0.5	0.5	0.5	0	0
herb	*Cardamine pratensis*	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0.1	0	0.1	0
herb	*Carex montana*	0.5	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	0	2
herb	*Carex nigra*	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	8	0
herb	*Cerastium alsinifolium*	0	1	0.1	0.5	0	0	0	0.1	0.1	0	0	0	0	0.1	0	0	0	0.5	0.1	0
herb	*Cirsium palustre*	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Dactylis glomerata*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Danthonia decumbens*	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Deschampsia cespitosa*	5	2	10	13	7	1	0.5	0.5	15	1	0.5	3	3	6	0	0	1	0	5	2
herb	*Dryopteris dilatata*	0	0	0	0	0.1	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0
herb	*Erica carnea*	0	6	1	0	0	0	17	0	0	0	0	0.1	0	0.5	0	0	0.1	2	0	0
herb	*Festuca ovina*	0	10	2	5	0.5	1	3	2	0	0	1	5	1	7	5	20	0.5	10	1	0.5
herb	*Festuca rubra*	2	0.5	5	7	5	0	0	0	3	1	0	0	1	0	0	0	0	0	0	15
herb	*Fragaria vesca*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Galium saxatile*	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1	0.1	0.1	0.1	0.5	0.5	0.5	5	0.5	0.5	0.5	0	0.5
herb	*Galium sudeticum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0.5	0	0.5	0	0
herb	*Galium verum*	0	1	0	0	0	1	2	0	0	0.5	0	2	0.5	1	0	0	0.5	0	0.5	0
herb	*Hieracium lachenalii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Hieracium pilosella*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Holcus mollis*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Juncus bulbosus*	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Larix decidua* (seedling)	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Lathyrus pratensis*	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0.1
herb	*Lathyrus sylvestris*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Leontodon hispidus*	0	8	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Lotus corniculatus*	0	0	0	0	0	0.5	0	0	0.1	0	0	0	0	0	0	0.1	0	0.5	0	0
herb	*Luzula pilosa*	0.5	2	1	0.5	0	0	0	0.5	0	0.5	0	0	0	0	0.5	0	0.5	0	0	0.5
herb	*Lychnis flos-cuculi*	0	0.5	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0.5	0
herb	*Melampyrum pratense*	0.5	1	0.5	0.5	0	0	0	0	0	0	0	2	0	0	0.5	0.5	0	0	0	0.5
herb	*Molinia caerulea*	0	15	0.1	2	0	10	5	0	1	15	35	0	0	12	0	0	20	0	9	0
herb	*Mycelis muralis*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Narduus stricta*	0	0.5	0	0	2	0.5	0	0	0	0	0	2	0	0	0	0	0	0	0.5	0

herb	*Orthilia secunda*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Oxalis acetosella*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Phleum bertolonii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Picea abies* (seedling)	0	0	0.5	0.5	0.1	0.1	5	0	0.1	0.5	0.1	0	0.5	0.1	0.1	0	0.1	0.5	0	0.5
herb	*Pimpinella major*	2	0.5	1	0	0	1	0	0	0	0	1	0.5	0.5	0	0	0	1	0	0	2
herb	*Pinus sylvestris* (seedling)	0	0.5	0	0	0.1	0	0.5	0.5	0.1	0	0	0	0.1	0	0	0	0	0.1	0.1	0.1
herb	*Plantago media*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Poa pratensis*	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Polygala chamaebuxus*	0.5	0	0.5	0	0	1	0.5	1	0	0	0	3	0	0	0.5	1	0.1	1	0	0
herb	*Polygala vulgaris*	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Potentilla erecta*	0.5	1	0.5	0.5	0.5	0.5	2	0.1	0.5	0	0.1	0.5	0.5	0.1	0	0.1	0.5	0.5	0.5	0.5
herb	*Prunella vulgaris*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Pteridium aquilinum*	25	0	20	70	0	0	0	0	15	0	0	0	0	0	0	0	20	3	0	20
herb	*Ranunculus acris*	0	0.5	0	0.5	0.5	0	0.5	0	1	0	0	0.5	0	1	0	0	0.5	0	0.1	0
herb	*Rosa canina* (juvenile)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Rubus idaeus*	0	0	0	0	0	0	0	0.1	0.5	0	0	0	0.1	0.1	0	0.1	0.1	0	0	0
herb	*Rumex acetosa*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0
herb	*Rumex acetosella*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0
herb	*Senecio ovatus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Silene vulgaris*	0	0.5	0.1	1	0	2	0.5	6	1	0.5	0.5	0	5	0	0	2	3	0	0.1	0.5
herb	*Sorbus aucuparia* (juvenile)	0	0	0	0	0	0.1	0.1	0	0	0	0.5	0.5	0.1	0.1	0	0	0	0	0	0.5
herb	*Stellaria alsine*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Stellaria graminea*	0	0.5	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Taraxacum* sect. *Ruderalia*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Tephroseris crispa*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
herb	*Thesium alpinum*	0	0	0	0	0	0.1	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Trientalis europaea*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Trifolium pratense*	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0
herb	*Trifolium repens*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Urtica dioica*	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herb	*Vaccinium myrtillus*	3	8	7	0.5	5	30	5	3	0.5	4	5	30	0	7	8	7	10	0	5	10
herb	*Vaccinium vitis-idaea*	0	2	1	0.1	0	3	3	0.5	0	0.5	0	0.5	0	0	0.1	0.1	0.5	0.5	0.5	0.5
herb	*Veronica chamaedrys*	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0
herb	*Veronica officinalis*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0

herb	*Vicia sepium*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5
herb	*Viola riviniana*	0	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
tree	*Picea abies*	69	0	0	15	8	1	0	4	40	38	40	0	40	15	1	25	3	1	5	0
tree	*Pinus sylvestris*	1	25	25	30	2	39	0	0	0	2	35	75	40	20	0	0	4	10	75	0
tree	*Larix decidua*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0

Appendix S2. (continued)

B

Environmental variable	Vegetation sample no.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Knautia cytotype	2x	2x	2x	4x	4x	4x	4x	2x	4x	2x	2x	4x	4x	2x	2x	2x	4x	2x	4x	2x
Mean EIV - Continentality	4.0	4.3	4.0	4.3	4.0	4.0	4.6	4.3	4.5	4.1	4.2	4.0	4.4	4.1	4.2	3.8	3.9	4.3	4.2	4.4
Mean EIV - Light	6.5	6.3	6.5	5.9	6.6	6.4	6.3	6.1	5.9	6.0	5.9	5.8	6.4	6.3	6.0	6.2	6.2	6.2	5.8	5.9
Mean EIV - Moisture	4.6	4.5	5.5	4.7	4.3	4.6	4.8	4.8	5.0	4.9	5.3	5.3	5.3	4.8	4.8	5.3	4.9	5.1	4.9	5.0
Mean EIV - Nutrients	3.3	3.2	2.9	3.4	2.9	3.2	4.1	2.8	3.4	3.0	3.4	3.7	3.0	2.5	3.1	3.1	3.0	3.6	3.1	3.1
Mean EIV - Reaction	4.1	3.9	3.9	3.9	4.3	4.7	4.9	4.4	3.5	3.6	3.9	3.9	4.1	3.6	4.4	4.2	4.1	4.4	4.2	4.0
Mean EIV - Temperature	4.2	4.1	3.9	4.0	4.1	3.9	4.0	3.7	3.9	3.5	3.7	4.1	4.1	4.0	3.7	4.2	4.0	3.8	3.5	3.8
Relative coverage - herb layer [%]	90	55	85	60	70	75	90	55	80	65	80	80	90	80	80	90	75	70	60	40
Relative coverage - moss layer [%]	94	75	60	75	75	90	50	70	95	80	95	50	70	65	50	40	80	85	95	90
Relative coverage - tree layer [%]	0	5	10	40	17	5	50	40	5	40	30	55	40	25	30	0	5	40	5	30
Slope inclination [°]	10	50	1	3	5	5	3	10	5	2	2	4	5	6	4	5	4	6	3	5
Species richness (no. of species)	33	21	27	28	28	30	24	25	31	22	29	30	25	25	26	23	27	28	27	29

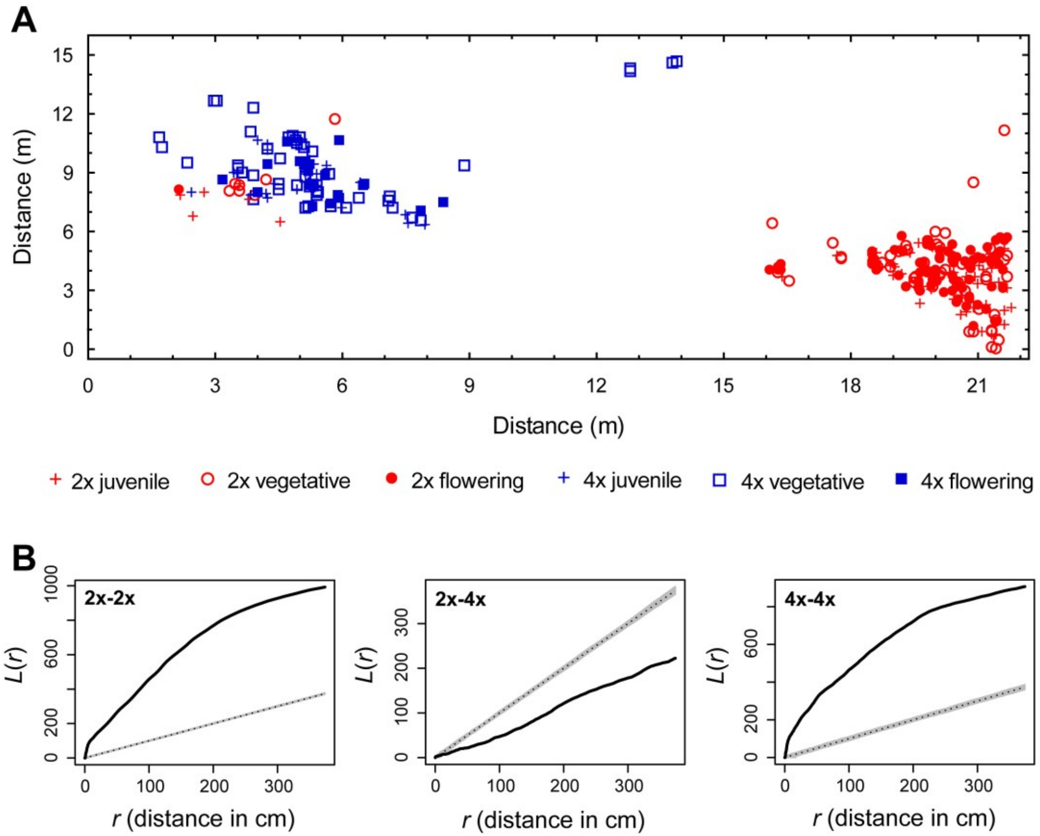
Note: EIV = Ellenberg indicator values (provide estimates of environmental characteristics of the sites inferred from species composition data)

Appendix S2. (continued)

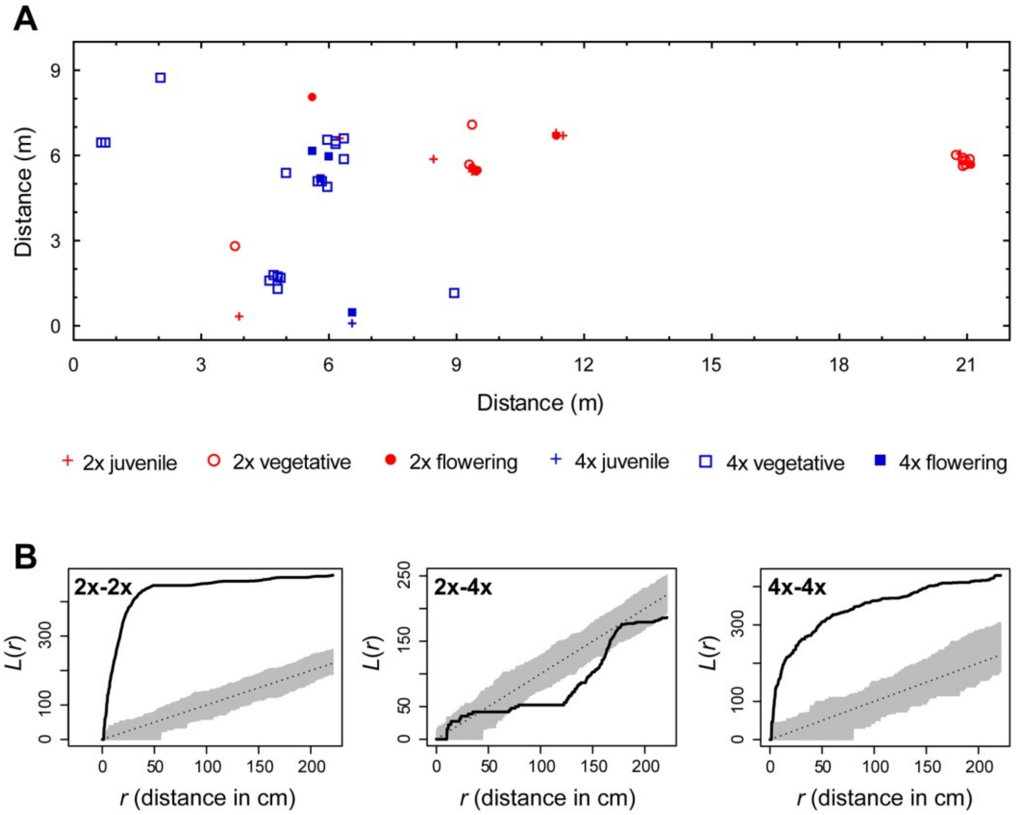
B

Environmental variable	Vegetation sample no.																			
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Knautia cytotype	4x	4x	4x	4x	2x	4x	2x	4x	4x	2x	2x	2x	2x	2x	4x	4x	2x	4x	2x	4x
Mean EIV - Continentality	4.3	3.8	4.1	4.3	4.6	4.1	3.9	4.4	4.6	4.6	4.4	4.0	4.4	4.5	4.4	3.9	4.1	4.3	4.4	4.2
Mean EIV - Light	5.8	6.5	6.1	6.0	6.2	6.5	6.5	5.8	6.3	5.7	6.1	6.4	6.2	6.2	5.8	6.2	6.0	6.2	6.5	6.0
Mean EIV - Moisture	4.9	5.4	4.9	5.4	5.4	5.0	4.6	4.8	5.2	5.0	5.4	4.7	5.4	5.3	4.5	4.4	5.0	4.4	5.5	5.2
Mean EIV - Nutrients	3.3	2.8	3.1	2.9	3.1	2.9	2.4	2.7	3.3	3.0	3.4	2.5	3.9	3.1	2.7	2.6	3.1	2.3	2.6	3.3
Mean EIV - Reaction	4.2	4.0	4.2	3.6	3.5	4.4	3.9	4.1	4.2	4.1	4.1	3.7	4.7	3.7	3.6	3.6	4.1	3.9	3.5	4.1
Mean EIV - Temperature	3.8	4.5	3.6	3.7	3.4	3.8	3.9	3.8	3.6	3.6	3.6	4.0	3.8	3.6	3.7	3.8	4.0	3.9	3.6	3.8
Relative coverage - herb layer [%]	45	90	75	95	55	70	70	40	70	40	50	70	50	65	35	50	60	70	45	75
Relative coverage - moss layer [%]	30	80	95	30	80	80	60	95	25	80	70	85	90	90	95	70	95	85	55	95
Relative coverage - tree layer [%]	70	25	25	45	10	40	0	4	40	40	75	75	80	35	5	25	7	11	80	0
Slope inclination [°]	3	4	4	2	3	3	4	30	5	3	2	4	2	6	15	7	5	7	4	6
Species richness (no. of species)	22	31	30	27	23	31	25	25	23	19	21	24	24	24	21	20	34	30	27	29

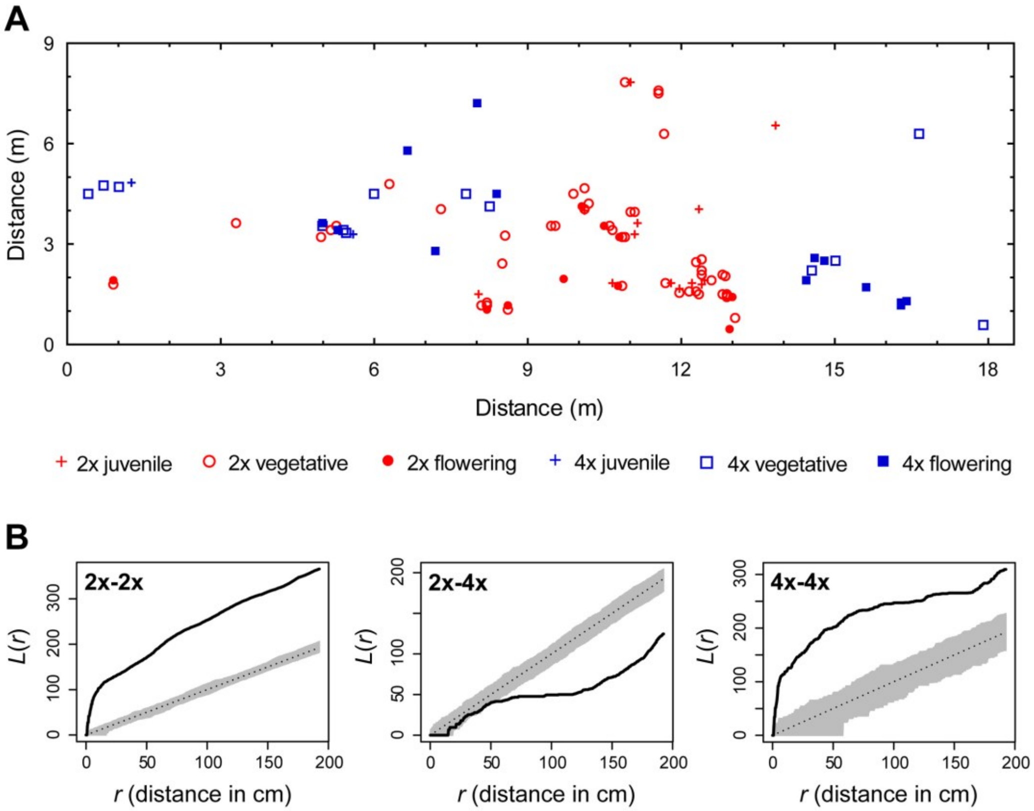
Note: EIV = Ellenberg indicator values (provide estimates of environmental characteristics of the sites inferred from species composition data)



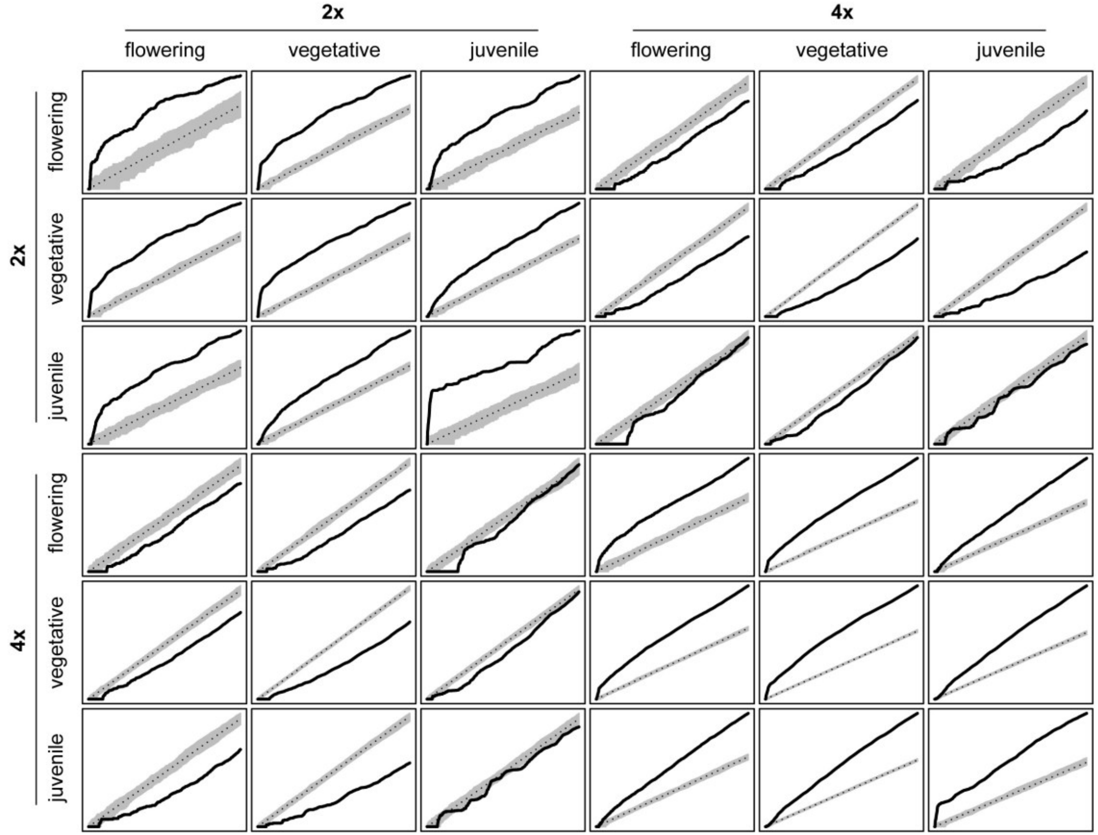
Appendix S3. Spatial positions of all *Knautia* individuals (divided into three life stages) in a mixed-ploidy plot M2 (A), and corresponding pair-wise inter- and intra-cytotype spatial associations (B). Expected values of the L(*r*) function are shown by the dotted line, 95% confidence interval shaded in grey. Values higher or lower than confidence limits indicate positive or negative associations, respectively.



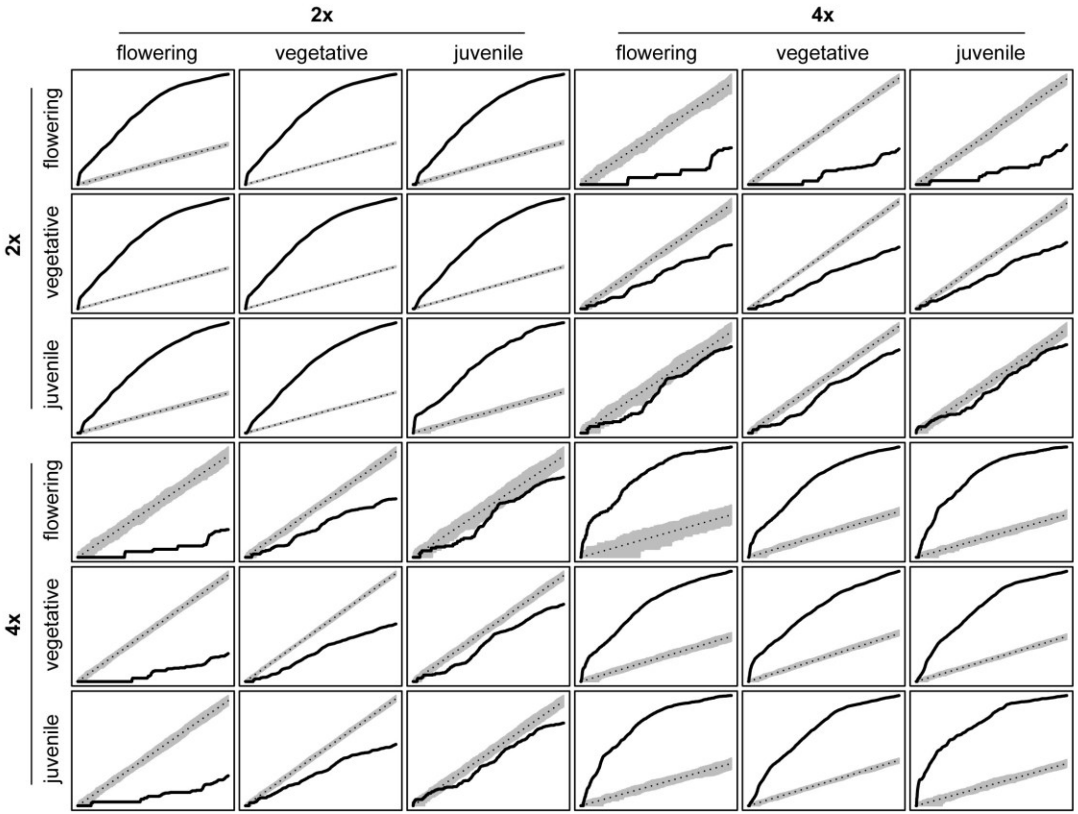
Appendix S4. Spatial positions of all *Knautia* individuals (divided into three life stages) in a mixed-ploidy plot M3 (A), and corresponding pair-wise inter- and intra-cytotype spatial associations (B). Expected values of the L(*r*) function are shown by the dotted line, 95% confidence interval shaded in grey. Values higher or lower than confidence limits indicate positive or negative associations, respectively.



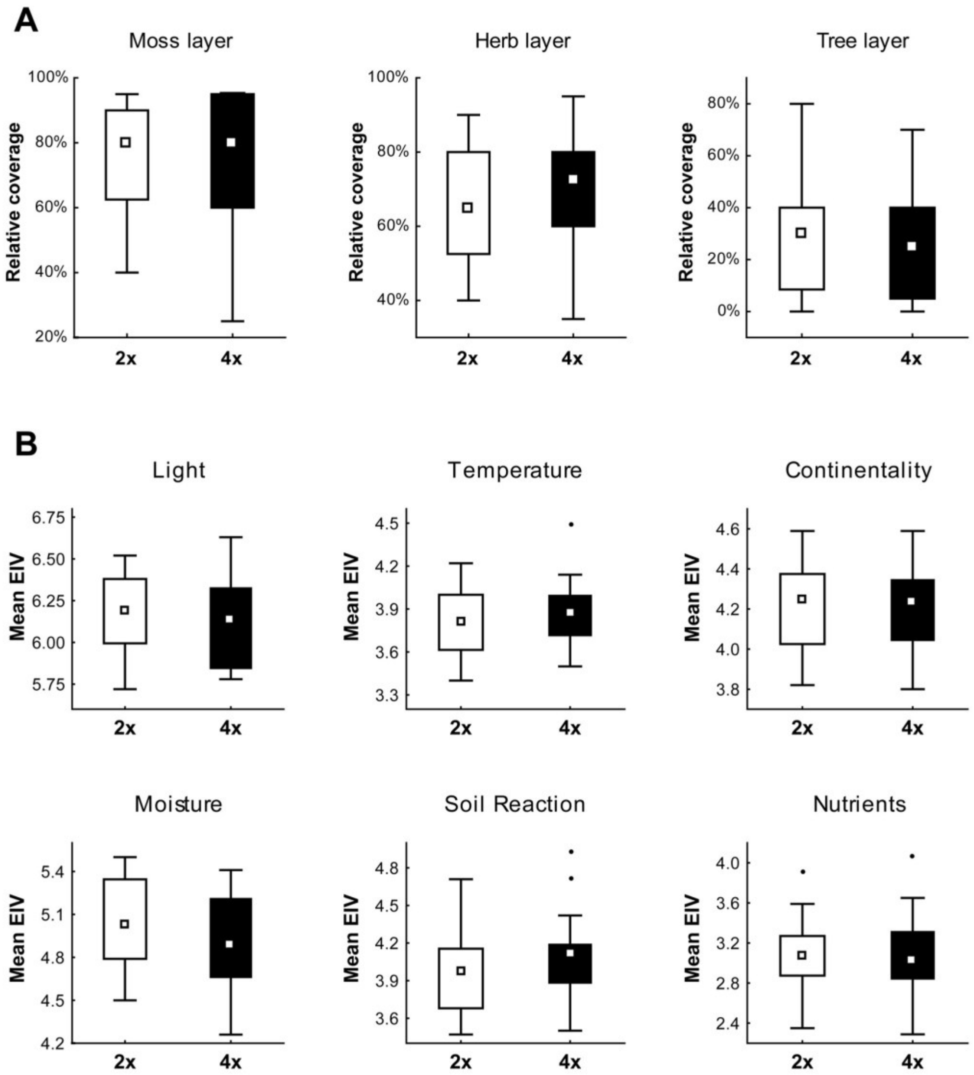
Appendix S5. Spatial positions of all *Knautia* individuals (divided into three life stages) in a mixed-ploidy plot M4 (A), and corresponding pair-wise inter- and intra-cytotype spatial associations (B). Expected values of the L(*r*) function are shown by the dotted line, 95% confidence interval shaded in grey. Values higher or lower than confidence limits indicate positive or negative associations, respectively.



Appendix S6. Pair-wise comparisons of spatial associations of di- and tetraploid *Knautia* individuals from mixed-ploidy plot M1 with respect to their life-stages. Expected values of the L(*r*) function are shown by the dotted line, 95% confidence interval shaded in grey. Values higher or lower than confidence limits indicate positive or negative associations, respectively.



Appendix S7. Pair-wise comparisons of spatial associations of di- and tetraploid *Knautia* individuals from mixed-ploidy plot M2 with respect to their life-stages. Expected values of the L(r) function are shown by the dotted line, 95% confidence interval shaded in grey. Values higher or lower than confidence limits indicate positive or negative associations, respectively.



Appendix S8. Environmental characteristics of 20 diploid (white) and 20 tetraploid (black) vegetation samples. (A) Relative cover of the three main vegetation layers (shrub layer was not developed). (B) Ellenberg indicator values (EIV) for six environmental variables inferred from species composition data.

PAPER II

Phenotypic consequences of genome doubling in stressful environments: Serpentine bedrock shapes the diploid – tetraploid coexistence in *Knautia serpentinicola* (Caprifoliaceae)

Experimental cultivation of *Knautia* diploids and their natural autotetraploid derivates under contrasting environmental treatments.

Phenotypic consequences of genome doubling in stressful environments: serpentine bedrock shapes the diploid – tetraploid coexistence in *Knautia serpentinicola* (Caprifoliaceae)

Martin Čertner[1,2], Radka Sudová[2], Martin Weiser[1], Jan Suda[1,2] & Filip Kolář[1,2,3]

[1]*Department of Botany, Faculty of Science, Charles University, Benátská 2, CZ-128 00 Prague, Czech Republic*

[2]*Institute of Botany, The Czech Academy of Sciences, Zámek 1, CZ-252 43 Průhonice, Czech Republic*

[3]*National Centre for Biosystematics, Natural History Museum, University of Oslo, NO-0318 Oslo, Norway*

Summary

Genome duplication is a key process in plant evolution with direct consequences on plant phenotypes. Yet it is still unclear, whether ploidy-related phenotypic changes can significantly alter the performance of polyploids in nature and thus contribute to establishment of new polyploid mutants in diploid populations.

We sampled a rare case of well-documented natural coexistence of recently formed autotetraploids with their diploid progenitors. Using a common garden experiment with two manipulated environmental factors (presence/absence of serpentine substrate and competition), we tested whether these two key factors shape differently the phenotypic and adaptive response of the two ploidy levels.

Tetraploid individuals attained significantly higher values of aboveground biomass, belowground biomass, and root/shoot ratio compared to their diploid progenitors. Tetraploid superiority in vegetative fitness indicators was most prominent when cultivated together with a competitor in nutrient-rich non-serpentine substrate.

Our results show that diploids and their autotetraploid derivatives differ in their phenotypic response to environmental factors. Provided enough nutrients, tetraploids can be more successful in tolerating interspecific competition than their diploid progenitors. Such superior performance might have allowed the tetraploids colonizing new sites, thus escaping competition and hybridization with diploids. This helps explaining the observed successful tetraploid establishment at the natural site.

Key words: mixed-ploidy population, nutrient uptake efficiency, polyploid establishment, polyploidization, primary contact zone, serpentine

INTRODUCTION

Polyploidization, the process of whole-genome duplication, is widely acknowledged as a major force in the evolutionary diversification of flowering plants (Soltis *et al.*, 2009; Wood *et al.*, 2009; Weiss-Schneeweiss *et al.*, 2013). All angiosperms have undergone one or more rounds of genome doubling in their evolutionary history (Wendel *et al.*, 2016) and present-day polyploid species even tend to have multiple origins (Soltis & Soltis, 1999). On the other hand, polyploid evolution is substantially constrained by overall low chances of polyploid mutants becoming established in populations of their diploid progenitors (Levin, 2002; Ramsey & Schemske, 2002). Given the generally low fitness of across-ploidy hybrids (Ramsey & Schemske, 1998), the initially rare novel polyploids will suffer from pollen swamping by diploids leading to less fit hybrid progeny and, ultimately, disappearance of the polyploid from such mixed-ploidy population (Levin, 1975; Husband, 2000). Nonetheless, several mechanisms have been suggested to compensate for the initial disadvantage of new polyploids in natural populations, allowing their establishment (Rodríguez, 1996; Husband, 2004; Yamauchi *et al.*, 2004; Baack, 2005; Suda & Herben, 2013). For example, within-ploidy pollen transfers may be increased due to ecological (spatial) segregation of the diploid and polyploid cytotypes (Lumaret *et al.*, 1987; Baack, 2004; Sonnleitner *et al.*, 2010), follow ploidy-specific differences in flowering phenology (Petit *et al.*, 1997; Nuismer & Cunningham, 2005) or distinct pollinator assemblages (Segraves & Thompson, 1999; Kennedy *et al.*, 2006). Reproductive assurance may also result from a shift towards autogamy (Barringer, 2007; Husband *et al.*, 2008).

Most of these mechanisms stem from ploidy-specific differences in particular plant traits that are traditionally attributed to the genome duplication *per se* (Levin, 1983, 2002). However, our understanding of the mechanisms of successful polyploid establishment is mostly based on studying the outputs of this process, i.e., by comparing long-established polyploids with their diploid relatives. Phenotypes of such "old" polyploids, however, could have been significantly shaped by local adaptation in allopatry and/or genetic drift (Petit *et al.*, 1999). Since the direct effects of genome doubling may be confounded with other changes gradually accumulating after the polyploidization event, the phenotypic and ecological consequences of genome duplication might be overestimated (Ramsey, 2011). More promising approaches to study phenotypic consequences of genome doubling are using synthetic polyploids (Husband *et al.*, 2008; Hegarty *et al.*, 2013) or spontaneously formed first-generation polyploid mutants obtained either from natural populations (Ramsey, 2011) or artificial crosses (Bretagnolle & Lumaret, 1995). However, even these methods share a common drawback – the first-generation polyploid offspring results from an arbitrary selection, which can significantly impact the phenotypic variation among new polyploids. A convenient alternative to these approaches is comparing biological properties of diploids and their sympatric, recently derived autopolyploids in so-called primary contact zones (e.g., Bretagnolle & Thompson, 2001; Šingliarová *et al.*, 2011; Hanzl *et al.*, 2014). The main advantage of primary contact zones is an opportunity to compare the two cytotypes under natural conditions, thus capturing only "biologically-relevant" variation. So far, however, only few studies addressed phenotypic consequences of genome doubling in the primary contact zones (Lumaret *et al.*, 1987; Maceira *et al.*, 1993).

We addressed phenotypic consequences of genome doubling in one of the few well-documented cases of a primary contact zone presented by diploid – autotetraploid populations of *Knautia serpentinicola* in western Czechia (Kolář *et al.*, 2012, Hanzl *et al.*, 2014). Natural setting provided by this species is optimal for our main aim, i.e. revealing the drivers of successful establishment of new polyploid mutants in natural populations. The two cytotypes are morphologically (Kaplan, 1998; Kolář *et al.*, 2015) and genetically (Kolář *et al.*, 2012) indistinguishable from each other, they also share similar habitat requirements (Hanzl *et al.*, 2014). Despite its local and presumably recent (postglacial) origin from a coexisting diploid race (Kolář *et al.*, 2012), the tetraploid is well established at the site and is even much more frequent than diploids. This points to existence of certain biological trait(s) that allowed the tetraploid to take over its initially dominating diploid progenitors.

Our previous *in situ* screening did not reveal any signs of ploidy-specific differences in habitat requirements at sites where diploids and tetraploids co-occur (Hanzl *et al.*, 2014). Since we originally omitted from ecological comparisons tetraploid-dominated peripheral parts of the locality, were environmental gradients are likely to occur, we now experimentally addressed the response of both cytotypes to two key environmental factors affecting plant life at the natural *K. serpentinicola* sites, i.e. specific substrate (serpentine) and competition with other plants. The species occurs exclusively at serpentine soils, substrates well-known to impose numerous challenges to plant life. Due to their low calcium to magnesium ratio, deficiency of essential macronutrients, increased (even toxic) concentrations of heavy metals, and low water-holding capacity, serpentine soils can be colonized only by (pre-) adapted biota (Proctor & Woodell, 1975; Brady *et al.*, 2005; Kazakou *et al.*, 2008). Although the previous hydroponic cultivation has shown similar response of both cytotypes to serpentine soil chemistry at the level of seedlings (Kolář *et al.*, 2014), the performance of adult plants in their native serpentine substrate was not known. Contrary to common theoretical expectations of better performance of polyploids under interspecific competition (Levin, 2002), this has only rarely been addressed experimentally and the available results are equivocal (Maceira *et al.*, 1993, Münzbergová, 2007, Collins *et al.*, 2011). A potential interaction between the competitive ability of cytotypes and abiotic environmental stress factors (such as those posed by serpentine soils) has not been studied at all.

By experimental cultivation of diploids and their recent tetraploid derivatives of *K. serpentinicola* we addressed the mechanisms facilitating successful polyploid establishment and cytotype coexistence in this species. Specifically, we asked following questions. (i) Are there any differences in fitness-related traits between the two cytotypes, resulting from genome doubling? (ii) Do the cytotypes differ in their phenotypic response to the two main environmental factors affecting plant growth at their native site (i.e. presence of inhospitable substrate and intensity of interspecific competition)?

MATERIALS AND METHODS

Study species and sampling

Knautia serpentinicola Smejkal ex Kolář, Z. Kaplan, J. Suda et Štech, a recently described member of the field scabious group (*Knautia arvensis* agg., Caprifoliaceae), inhabits open pine forests on four isolated serpentine outcrops in Central Europe (Kolář *et al.*, 2015). While three of these localities are occupied by exclusively diploid (2n = 2x = 20) populations, one in the Slavkovský les Mts. (W Bohemia, Czech Republic) is mixed-ploidy, and aside from diploids harbours also individuals of an autotetraploid cytotype (2x = 4x = 40; Kolář *et al.*, 2009). Both diploids and tetraploids are genetically undistinguishable (Kolář *et al.*, 2012). The species is a rhizomatous perennial herb with a well-developed basal leaf rosette and sparsely branched leafy stems of height up to 60 cm. Pink to deeply purple tetramerous flowers are clustered in terminal flower heads and are pollinated by insect. The plants spread either by ant-distributed one-seeded achenes or through vigorous clonal growth based on rhizome elongation and formation of accessory leaf rosettes (Štěpánek, 1997). Previous work has shown that both cytotypes of *K. serpentinicola* are well adapted to harsh conditions of serpentine sites by their physiology (Kolář *et al.*, 2014) and symbiosis with arbuscular mycorrhizal fungi (Doubková *et al.*, 2012).

Plant material originated from the single mixed-ploidy *K. serpentinicola* locality in the Slavkovský les Mts. (50°02'19"N, 12°44'59"E). In 2011, we collected ripe achenes (for simplicity called “seeds” hereafter) in the four most abundant ploidy-uniform subpopulations of diploid and tetraploid cytotype (Supporting Information Table S1). Additionally, we collected seeds of local serpentine-tolerant genotype of *Agrostis capillaris* L. (Poaceae) to be later used as plant competitor in the experiment. Serpentine soil for cultivation was sampled from four sites inhabited by mixed-ploidy subpopulations of *Knautia*, pooled together and homogenized, so neither ploidy level nor pertinent ecotype of residing subpopulations could be favoured in the experiment.

Seed germination

In total, 726 diploid and 890 tetraploid well-developed seeds were subjected to controlled germination. The seeds were pooled by their subpopulation of origin and put in 10-cm-diameter Petri dishes inlaid with cellulose, reaching a maximal density of 50 seeds per dish. Stable conditions during experimental germination were controlled using Forma Diurnal Growth Chamber (Thermo Scientific, Waltham, USA) under the following setting: 14 h light, 13 °C; 10 h dark, 13 °C. The Petri dishes were watered regularly and a number of germinating seeds (i.e., with >2-mm-long radicle) was recorded in 2-3 day intervals. After 37 days, when seed germination rates became constant, the germination was terminated and a subset of seedlings was used in a follow-up cultivation.

Experimental cultivation

For cultivation, we chose 110 well-developed seedlings of each ploidy level using stratified random sampling to cover representatively various subpopulations of seed origin (see Supporting Information Table S1). To check for any bias during the selection process, length and width of cotyledons were measured for all the remaining well-developed seedlings not entering the study (164 in sum) and their values were compared with the selected individuals. Seedlings were transplanted to 1.4 L plastic pots and cultivated under greenhouse conditions at the experimental garden of Institute of Botany, The Czech Academy of Sciences (approx. 130 km apart from the original sites) from April to August in 2012. The experiment combined three independent factors in a full-factorial design (see Fig. 1): ploidy level of *Knautia* seedlings (2x, 4x), type of substrate (serpentine soil, common garden soil) and interspecific competition (presence or absence of a competitor). The serpentine-substrate treatment consisted of a 2 cm-high layer of serpentine gravel on the bottom of each pot topped with fine-grained serpentine soil, as this arrangement best reflected the natural conditions at the sampled *Knautia* sites. Serpentine-tolerant genotype of grass *Agrostis capillaris*, a frequent dominant at *Knautia*-inhabited sites (Hanzl *et al.*, 2014), was used to infer interspecific competition. In this treatment, single *Knautia* seedling was transplanted to a pot with two-week-old *Agrostis* seedlings.

Pots were randomly shuffled each week to minimize the effect of uneven light and irrigation intensities. We checked the ploidy level of all plants after three months of cultivation using flow cytometry, following our protocol previously used in this species (Hanzl *et al.*, 2014). In all cases the estimated ploidy level corresponded and was shared by all individuals originating from the same subpopulation. The experiment was terminated after 137 days, when plants from the control treatment stopped exhibiting signs of growth. None of the cultivated plants reached flowering stage, which is in agreement with our field observations of earliest flowering in the second year of plant life. Four vegetative fitness indicators were scored on each cultivated *Knautia* individual: length of the largest leaf, width of the largest leaf, number of leaf pairs and number of accessory leaf rosettes. All *Knautia* individuals were harvested (the above- and below-ground parts separately), their biomass dried at 80°C and weighted. The belowground biomass could not be assessed for plants grown with a competitor because we were unable to separate the roots of *Knautia* from those of *Agrostis*.

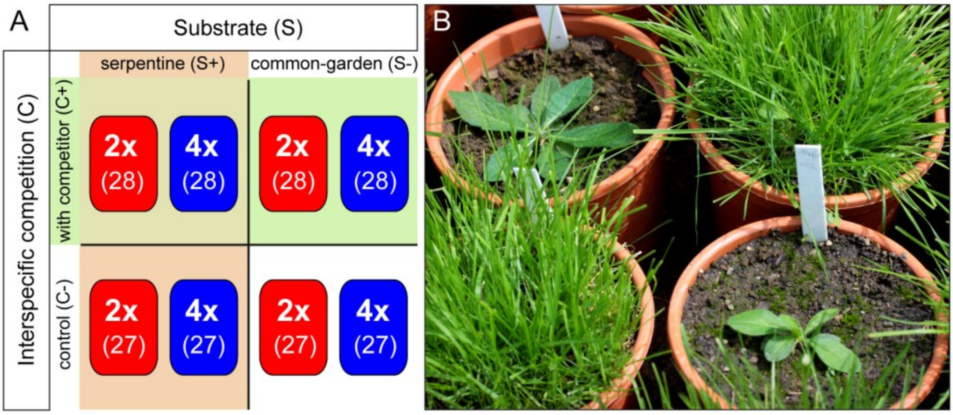


Fig. 1 Experimental design combining three factors (interspecific competition, substrate quality, and ploidy level) in a full-factorial manner (A), with numbers of individuals subjected to each treatment stated in brackets. Four Knautia individuals on a snapshot from the 41st day of the experiment (B), two of the plants were grown solitarily and two with an interspecific competitor.

Soil analysis

We took soil samples of serpentine and common garden substrates both before and after the experimental cultivation. Random subsamples were always taken across at least 20 pots, homogenized and air-dried. The soil samples were then passed through a 2-mm sieve and analyzed for pH, net dry weight, nutrient and metal concentrations. The contents of N, total C, and organic C (the latter after an HCl treatment) were inferred through combustion in a Carlo Erba NC 2500 elemental analyzer (Carlo Erba, Milan, Italy). The Mehlich III reagent (Mehlich, 1984) was used to extract other macronutrients (P, Na, K, Ca, and Mg). The concentration of available P was inferred in reaction with $(NH_4)_6Mo_7O_{24}$ using a Unicam UV4-100 spectrophotometer (ATI Unicam, Cambridge, UK). Concentrations of available Na, K, Ca, and Mg were estimated using an AAS spectrometer ContrAA 700 (Analytik Jena, Jena, Germany), the latter two after a combined treatment of H_2SO_4 and La_2Cl_3. Concentrations of Ni, Co, Cr, and Fe were assessed by AAS spectrometry after DTPA-TEA soil extraction at pH 7.3, following Lindsay & Norvell (1978).

Data analysis

All statistical analyses were performed in R software ver. 3.1.2 (R Core Team, 2014). An onset of germination (i.e., the time necessary for the first seeds to germinate) and a final germination percentage were used to characterize the progress of seed germination. Ploidy-specific differences in population means of these variables were tested in an analysis of variance (ANOVA).

Indicators of plant fitness recorded at the end of experimental cultivation, i.e. values of four traits scored on *Knautia* individuals and their harvested biomass, were analyzed using ANOVA and generalized linear models (GLM) with the three factors

(Ploidy – diploid/tetraploid, Substrate – serpentine/non-serpentine, Interspecific competition – with/without a competitor) as explanatory variables. The measurements of length and width of the largest leaf were multiplied to produce an approximate leaf size, which was then used in all statistical analyses. The differences in largest-leaf size, number of leaf pairs, aboveground biomass, belowground biomass, total plant biomass, and root/shoot ratio (i.e., the proportion of belowground and aboveground biomass) across the experimental treatments were tested separately using univariate ANOVA models followed by Tukey's HSD post hoc tests. All the response variables except number of leaf pairs were transformed (log, square root) to meet the model assumptions. Due to lack of data on belowground biomass for plants grown in association with a competitor, the analyses of belowground biomass, total biomass, and root/shoot ratio were made on a reduced dataset consisting of 101 individuals cultivated without a competitor. Number of accessory leaf rosettes was analyzed using GLM of Poisson family. Significance levels were assessed using Chi test statistics. Pair-wise multiple comparisons of means with Tukey contrasts available in the R package "multcomp" ver. 1.4-6 (Hothorn *et al.*, 2008) were used as a post hoc test.

In order to identify the main cause(s) of significant changes in plant aboveground biomass among the experimental treatments, the relative importance of four traits scored on *Knautia* individuals was assessed. A linear regression model was fitted with the aboveground biomass as a response variable and an optimal set of predictors chosen during AIC-based selection from their pool: leaf length, leaf width, leaf length × width, no. of leaf pairs, and no. of accessory leaf rosettes. Most of the variables were square-root transformed (all except leaf length) to improve their normality. The relative contribution of particular predictors in the final model was assessed by method of averaging over orderings of regressors (following Lindemann *et al.*, 1980) using tools from the R package "relaimpo" ver. 2.2-2 (Grömping, 2006).

RESULTS

Seed germination

Over the course of 37 days of the experiment, 349 diploid and 475 tetraploid seeds germinated, constituting 48.1% and 53.4% of the total number of seeds of respective cytotypes. Seeds of diploids and tetraploids did not differ in onset of germination ($F_{1,6} = 1.321$, $P = 0.294$) nor in final germination rates ($F_{1,6} = 3.415$, $P = 0.114$).

Ploidy-driven differences in plant phenotypes

At the end of experimental cultivation, on the 137th day since planting, significant ploidy-specific differences were identified for all the phenotypic traits measured on *Knautia* individuals (Tab. 1). Most importantly, tetraploids attained higher mean values of both aboveground and belowground biomass, resulting in

Table 1 The effect of three experimental treatments and their interactions on each of seven traits scored on *K. serpentinicola* individuals after 137 days of cultivation. Significance was tested either using three-way ANOVA models (A) or using GLM of Poisson family, when normality of the data could not be assumed (B).

(A) Plant trait	Ploidy (P)			Substrate (S)			Competition (C)			P × S			P × C			S × C			P × S × C		
	d.f.	F	P	d.f.	F	P	d.f.	F	P	d.f.	F	P	d.f.	F	P	d.f.	F	P	d.f.	F	P
Largest leaf size	1,196	40.9	***	1,196	75.9	***	1,196	367.7	***	1,196	13.8	***	1,196	7.8	**	1,196	3.2	·	1,196	5.2	*
No. of leaf pairs	1,196	4.8	*	1,196	3.5	·	1,196	625.7	***	1,196	0.9	n.s.	1,196	3.8	·	1,196	1.2	n.s.	1,196	0.5	n.s.
Aboveground biomass	1,196	21.0	***	1,196	67.0	***	1,196	961.6	***	1,196	8.9	**	1,196	2.4	n.s.	1,196	15.0	***	1,196	3.7	·
Belowground biomass[†]	1, 97	37.6	***	1, 97	5.7	*	–	–	–	1, 97	0.0	n.s.	–	–	–	–	–	–	–	–	–
Total biomass[†]	1, 97	31.6	***	1, 97	20.2	***	–	–	–	1, 97	0.1	n.s.	–	–	–	–	–	–	–	–	–
Root/shoot ratio[†]	1, 97	30.0	***	1, 97	5.0	*	–	–	–	1, 97	0.2	n.s.	–	–	–	–	–	–	–	–	–

***P < 0.001, **P < 0.01, *P < 0.05, ·P <0.1

[†] The estimates of belowground biomass were not available for plants cultivated under interspecific competition (C) treatment, resulting in a reduced dataset of 101 individuals.

(B) Plant trait	Ploidy (P)			Substrate (S)			Competition (C)			P × S			P × C			S × C			P × S × C		
	d.f.	D	P	d.f.	D	P	d.f.	D	P	d.f.	D	P	d.f.	D	P	d.f.	D	P	d.f.	D	P
No. of leaf rosettes	1,218	8.2	**	1,217	19.4	***	1,216	329.0	***	1,215	2.6	n.s.	1,214	1.4	n.s.	1,213	0.5	n.s.	1,212	1.1	n.s.

***P < 0.001, **P < 0.01, *P < 0.05.

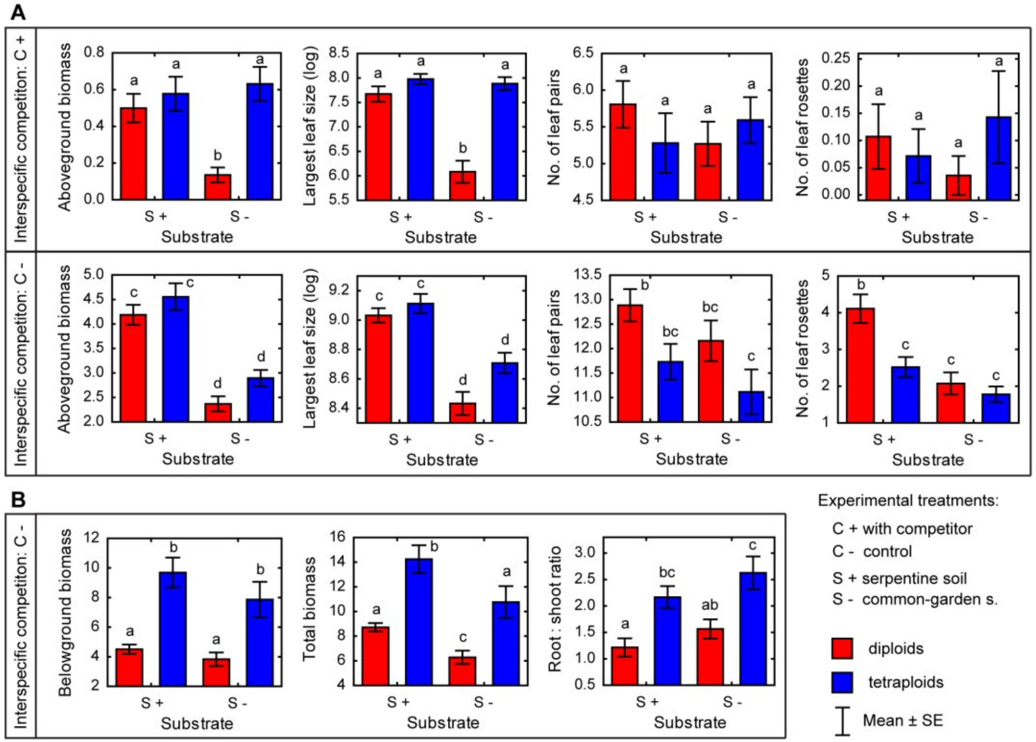


Fig. 2 The mean values of seven traits scored on *Knautia* individuals after 137 days of cultivation. Diploid and tetraploid individuals were subjected to different levels of two key environmental factors affecting plant life at their native site: substrate quality and interspecific competition. The effect of all treatment combinations is presented for aboveground biomass and its three predictors (A), however, from technical reasons, only estimates from cultivation without the competitor are available for belowground biomass and two related traits (B). For each trait separately, different letters indicate significantly ($\alpha = 0.05$) different effects of treatment combinations in pair-wise comparison in post hoc tests. Note the different y-axis range for the plants in and out of competition.

a substantial increase in total plant biomass when compared to their diploid counterparts. This trend was apparent across all experimental treatments (Figs. 2A, B). The tetraploids also showed higher root/shoot ratios (i.e., the proportion of belowground and aboveground biomass) and thus proportionally higher investment into belowground plant organs, irrespective of the type of cultivation substrate (Fig. 2B). Tetraploid individuals had significantly higher values of largest-leaf size but lower number of leaves and of accessory leaf rosettes than diploids (Tab. 1, Fig. 2A), suggesting that the higher aboveground biomass of tetraploids might stem solely from an increase in leaf size. All these traits, measured at the end of the experiment right before biomass harvest, were retained as predictors in the optimal linear model, which explained altogether 90% of variability in aboveground biomass (adj. $R^2 = 89.8\%$). Largest-leaf size was the best proxy of aboveground biomass (36.4% of total variation), followed by no. of leaf pairs (27.6%) and no. of accessory leaf rosettes (25.9%). Interestingly, tetraploid seedlings entering the experimental cultivation already had significantly larger cotyledons (mean = 67.9 mm^2 ± 1.6 SE) than their diploid counterparts (mean = 59.4 mm^2 ± 1.4 SE; $F_{1, 218} = 16.5$, $P < 0.001$). This difference could not be attributed to a biased selection of seedlings used for the experiment as the trait values of cultivated seedlings completely fell within the variability observed among the non-selected seedlings (pair-wise comparisons in a Tukey's HSD post hoc test: $P = 0.717$ and $P = 0.999$ for diploids and tetraploids, respectively).

The effect of native serpentine substrate

In an analysis of soil samples, we detected calcium-to-magnesium ratios of 0.2 and 13.9 for serpentine and common-garden substrates, respectively. The serpentine substrate also displayed strikingly lower concentrations of available phosphorus and potassium but elevated levels of phytotoxic heavy metals (nickel and cobalt especially) when compared to the common-garden substrate (see Supporting Information Table S2). Plant performance in their native serpentine substrate differed from that in common garden soil in all but one trait (marginally non-significant for no. of leaf pairs; Tab. 1). *Knautia* individuals cultivated in serpentine soil generally displayed higher mean values of fitness indicators, and these differences were especially pronounced for aboveground biomass and one of its predictors (largest-leaf size; Fig. 2A). However, the substantial increase in aboveground biomass was followed by only a smaller, disproportional, increment of belowground biomass, resulting in lower mean values of root/shoot ratio among the plants cultivated in serpentine substrate (Fig. 2B).

Interestingly, the plant response to substrate was ploidy-specific (i.e., significant Ploidy × Substrate interaction) for aboveground biomass and largest-leaf size (Tab. 1), resulting in more prominent differences between generally smaller diploids and larger tetraploids in these traits when grown in a common garden substrate (Fig. 3A).

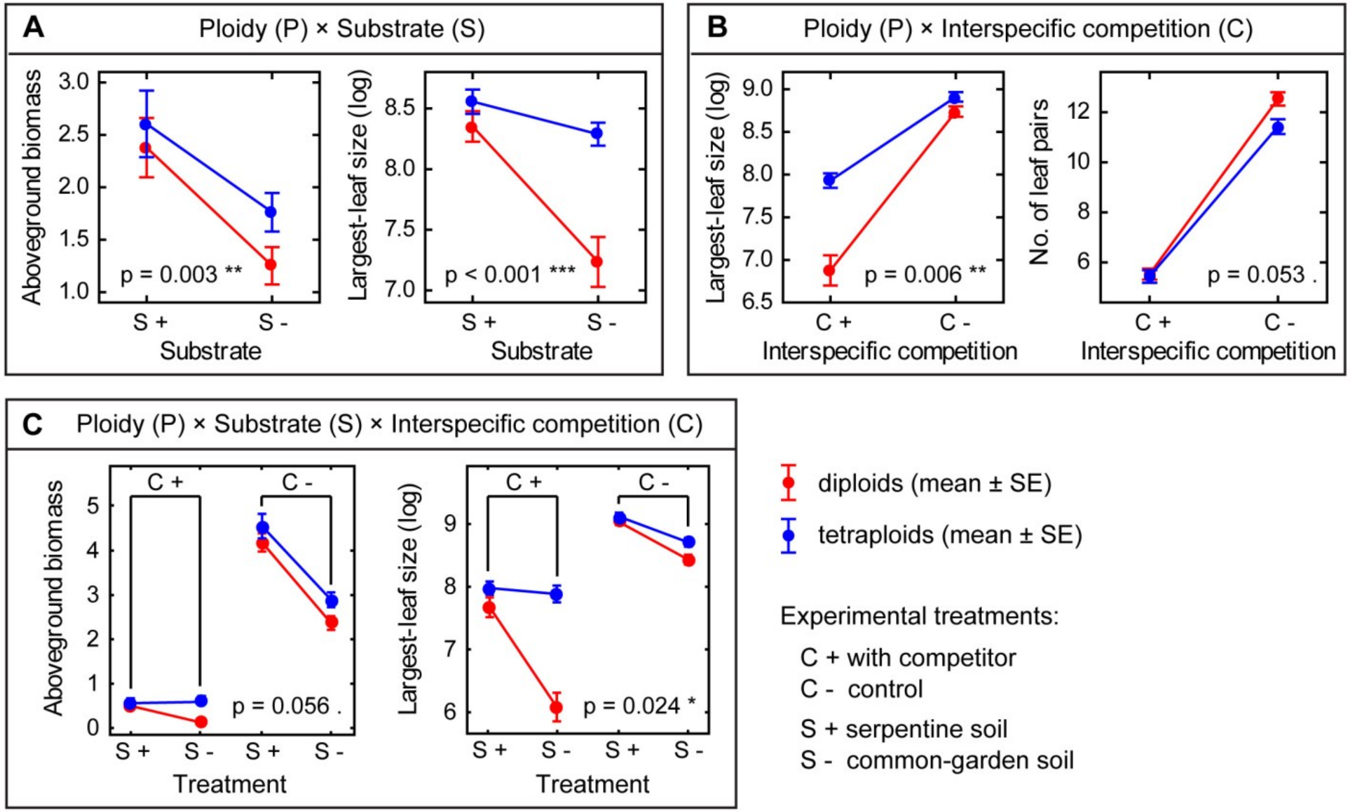


Fig. 3 Mean ± SE values of fitness indicators illustrating interactions of ploidy level of *Knautia* individuals with two environmental factors in cultivation: soil quality and interspecific competition. Only traits with significant (or marginally non-significant) interactions are presented here.

The opposite trend was recorded for the number of accessory leaf rosettes when plants were cultivated without an interspecific competitor (Fig. 2A), albeit Ploidy × Substrate interaction was not significant in this case.

Growth with an interspecific competitor

Competition with the major local competitor (grass *Agrostis capillaris*) significantly influenced values of all measured traits on *Knautia* individuals (Tab. 1). This treatment had the most profound effect on plant phenotypes, resulting in reduction of number of leaves, reduced leaf size, and in a virtual absence of accessory leaf rosettes, thus markedly decreasing the overall amount of plant aboveground biomass (Fig. 2A). However, interspecific competition did not lead to higher plant mortality, as only 8 (62%) of the plants that perished on the course of the experiment underwent this treatment.

The interaction of Ploidy (P) × Substrate (S) × Interspecific competition (C) was significant for largest-leaf size and marginally non-significant for aboveground biomass (Tab. 1), as tetraploids showed substantially higher values of these fitness indicators compared to their diploid progenitors when cultivated together with a competitor in common-garden substrate (Fig. 3C). The P × C interaction was significant for largest-leaf size and marginally non-significant for number of leaf pairs (Tab. 1). Tetraploids had larger leaves than diploids, in particular when grown with a competitor but the opposite pattern was observed for the number of leaf pairs (diploids with more leaf pairs than tetraploids, in particular outside competition Fig. 3B). The highly significant S × C interaction for aboveground biomass (and marginally non-significant for largest-leaf size; Tab. 1) is in line with the less pronounced phenotypic effect of substrate type on *Knautia* individuals cultivated with a competitor (Fig. 2A).

DISCUSSION

Phenotypic consequences of genome doubling

Phenotypic changes arising as a direct consequence of the polyploidization event are frequently discussed, however only rarely empirically documented drivers of successful establishment of new polyploid mutants in natural populations (Levin, 2002; Ramsey & Schemske, 2002; Parisod *et al.*, 2010). Ploidy-driven phenotypic variation is mostly inferred from comparisons between long-established polyploids and their genetically distinct diploid relatives (Petit *et al.*, 1999; Ramsey & Ramsey, 2014). However, in such cases the effect of genome doubling *per se* is largely obscured by subsequent changes gradually accumulating after the polyploidization event. Consequently, by studying such divergent di-polyploid systems, we may overestimate the extent to which genome duplication alters fitness-related plant traits and promotes success of newly-originated polyploids.

The recently derived tetraploid cytotype of *K. serpentinicola*, naturally coexisting with its genetically nearly identical diploid progenitor (Kolář *et al.*, 2012, 2015; Hanzl *et al.*, 2014) provides a unique opportunity to address the consequences of genome duplication for polyploid establishment. In our experimental comparison of the two sympatric cytotypes, tetraploid plants consistently displayed significantly higher values of both aboveground and belowground biomass (Fig. 2). Out of three traits measured on plants before harvest, tetraploids only had higher mean values of largest-leaf size and this trait also proved to be the most reliable proxy of aboveground biomass. The increase in the aboveground biomass could therefore be likely attributed to increments in leaf size. Interestingly, ploidy-specific differences in leaf size emerged early in the *Knautia* ontogeny and were already apparent at the stage of freshly potted seedlings. This was neither due to a bias in initial selection of the experimental plants nor a consequence of different timing of germination. The larger plant biomass will thus likely be a specific feature of tetraploids' phenotypes throughout the entire period of their vegetative growth.

Similar trends were commonly observed both in natural populations of other heteroploid model systems (e.g., Petit *et al.*, 1997; Gauthier *et al.*, 1998; Keeler & Davis, 1999; Kim *et al.*, 2012) and in synthetic polyploids (Levin, 2002; Hegarty *et al.*, 2013). In our case, given the relatively recent origin of *K. serpentinicola* tetraploids from the diploid cytotype, genome doubling *per se* has more likely caused the observed phenotypic changes than selection and/or other sources of cytotype diversification. The larger leaves of tetraploids could be a consequence of the so-called "gigas effect" (Stebbins, 1971), an increase in polyploid cell size that often translates into increased organ size and greater overall plant biomass. This is in agreement with our results showing significant increase with ploidy only for traits depending on cell size (leaf length, leaf width) but not for traits reflecting rate of cell division (timing and progress of germination) or an overall plant architecture (number of leaves, number of accessory leaf rosettes).

Aside from aboveground biomass, the tetraploid individuals also had significantly higher values of belowground biomass and, more importantly, higher root/shoot ratios suggesting their proportionally higher investment into belowground organs. Interestingly, the higher root/shoot ratio of tetraploid plants was observed both in nutrient-rich and nutrient-poor substrate settings (Fig. 2B), indicating genetic basis of this trait. Generally, higher root/shoot ratio is a common adaptation among serpentine-tolerant plant species, helping them to cope with the stressful conditions of their native soil (Proctor & Woodell, 1975; Brady *et al.*, 2005; Kazakou *et al.*, 2008). However, in our case the substrate-driven local adaptation would have to act more strongly on tetraploid than on diploid *Knautia* individuals, which is less likely for two reasons. Firstly, the tetraploids co-occur there alongside with diploid progenitors, and both cytotype distribution patterns and ecological comparisons suggest they face the same or even lower levels of serpentine-induced stress (Hanzl *et al.*, 2014). Secondly, according to teoretical expectations, selection should be less efficient in polyploid populations compared to diploid ones due to longer time to alelle fixation and this is especially the case of young autopolyploid derivatives with polysomic inheritance (Parisod *et al.*, 2010). Alternatively, if dried biomass of root (and rhizome) tissue was heavier than dried biomass of leaf tissue per cell, an increase in tetraploid cell size could also be responsible for higher root/shoot ratio, making it a direct consequence of polyploidization. The scarce available evidence seem to be rather species-specific, documenting both higher and lower investment of polyploids below ground relative to diploids (Sudová *et al.*, 2010; Collins *et al.*, 2011) or, alternatively, lack of ploidy-specific differences (Baack & Stanton, 2005).

Phenotypic novelty in the context of environmental variation

After 137 days of experimental cultivation, significant differences between diploids and tetraploids were observed for all recorded phenotypic traits, with total biomass (larger in tetraploids) exhibiting the strongest difference (Fig. 2B). While this is in concert with the findings from studies of synthetic polyploids (Levin, 2002; Hegarty *et al.*, 2013), it is still unclear, whether these differences could significantly alter the performance of cytotypes under natural conditions. The plants in natural populations are exposed to many environmental factors and ecological processes, which might differentially interact with ploidy-induced phenotypic variation. Natural diploid – autopolyploid populations (i.e. primary contact zones; Petit *et al.*, 1999) thus provide a convenient alternative to synthetic polyploids when assessing the response of cytotypes to local environmental conditions. In this study, we experimentally exposed the diploids and tetraploids of *K. serpentinicola* to two major ecological factors affecting plant growth at the site of their origin: interspecific competition and serpentine substrate.

Both factors had significant effect on *Knautia* performance. Plants cultivated in association with the grass *Agrostis capillaris*, a common dominant of most *Knautia*-inhabited sites (Hanzl et al., 2014), showed marked decrease of aboveground biomass (and of all its indicators; Fig. 2A). Individuals cultivated in serpentine soil attained higher values of total plant biomass (and of other indicators of plant vigor) but lower root/shoot ratios than their counterparts from common-garden substrate (Fig. 2). A positive effect of serpentine substrate on growth of serpentine-tolerant species was occasionally reported, although most of such plants fares better in nutrient-rich substrates (Brady *et al.*, 2005). Theoretically, if the common-garden substrate used in our study was somehow suboptimal, e.g. due to a lack of suitable mycorrhizal symbionts present in their native soil, *Knautia* individuals might grow less intensively and/or invest more resources into extensive root system. Nevertheless, we can rule out the simple absence of mycorrhizal symbionts in common-garden substrate, as root systems of ten randomly sampled plants from each substrate type were all colonized by mycorrhizal fungi (R. Sudová, unpublished results).

Although both ploidy level and environmental factors significantly shaped the individual phenotypes of *K. serpentinicola* in our experiment, it is the divergent performance of cytotypes under particular environmental settings that might unveil the ecological consequences of genome doubling. Interestingly, although tetraploid individuals exhibited higher values of aboveground biomass (and of largest-leaf size) across all the experimental treatments, the tetraploid advantage was more prominent when plants were cultivated in nutrient-rich common-garden substrate than in their native nutrient-poor and toxic serpentine soil (i.e. significant Ploidy × Substrate interaction; Fig. 3A). The differences between diploids and tetraploids were even more pronounced when, alongside of growing in common-garden substrate, the plants also experienced interspecific competition (Figs. 2A, 3C), though the corresponding interaction of Ploidy × Substrate × Interspecific competition was significant only for largest-leaf size and marginally non-significant for aboveground biomass. Given that plant size (measured e.g. in biomass production) and leaf area (largest-leaf size) are important predictors of plant competitive ability (Gaudet & Keddy, 1988; Keddy *et al.*, 2002), our results suggest that provided enough nutrients, tetraploids can be more successful in tolerating interspecific competition than their diploid progenitors.

We are fully aware that our experimental design did not allow for direct assessment of the strength of inter-cytotype competition, which would be possible only if we grew diploid and tetraploid individuals together (Maceira *et al.*, 1993; Collins *et al.*, 2011). However, we are convinced that the chosen design was more appropriate for *K. serpentinicola*. The incidence of mixed-ploidy patches, with diploid and tetraploid individuals co-occurring in close proximity, is quite uncommon at the locality (Hanzl *et al.*, 2014) and the two cytotypes thus rather face competition with other plant species than with each other.

Consequences for polyploid establishment and cytotype coexistence

New polyploid mutants emerging in natural populations of their diploid progenitors face a considerable constraint to their establishment (Husband, 2000). This stems not just from the direct competition between the two cytotypes (Yamauchi *et al.*, 2004) but mainly from a frequency dependent selection caused by low offspring fitness in inter-ploidy crossings (Levin, 1975). In *Knautia*, the observed greater total biomass of tetraploids and their higher tolerance to interspecific competition in nutrient-rich substrate might have compensated for their initial minority disadvantage, resulting in the current dominance of tetraploids at the locality. Theoretical models show that substantially higher vigour of polyploids may increase the probability of their establishment (Felber, 1991; Rodríguez, 1996). However, polyploids may also escape the problem by colonizing new sites, if they can better tolerate interspecific competition and/or efficiently use nutrients under some (micro-)habitat conditions (Fowler & Levin, 1984; Lumaret *et al.*, 1987; Sonnleitner *et al.*, 2010). This is, indeed, likely in *K. serpentinicola* whose tetraploids have historically expanded within the serpentine area, from sparse and long-term stable forests (where they only co-occur with diploids) towards newer forest plantations (Hanzl *et al.*, 2014). It was reported earlier from the area that such plantations may lead to accumulation of litter and subsequent increase in nutrient levels, locally buffering the effect of serpentine soil (Jeník, 1994). The observed better performance of *K. serpentinicola* tetraploids under high-nutrient high-competition conditions thus might have enabled their expansion to human-affected and more nutrient-rich sites.

A question rises whether our conclusions could have even more general consequences, e.g. by suggesting superior resource utilization by polyploids in nutrient-rich substrates as a direct consequence of genome doubling. Some support for this hypothesis can be found in early studies (reviewed by Levin, 1983) and also among recent work (e.g. Chao *et al.*, 2013) suggesting higher nutrient-uptake efficiency of polyploids when compared to diploids. Also a long-term application of phosphorus to oligotrophic grasslands substantially increased the relative success of polyploids at particular sites (Šmarda *et al.*, 2013; Guignard *et al.*, 2016). On the other hand, diploid and tetraploid *Dactylis glomerata* from a primary contact zone in NW Spain did not show significant differences in cytotypes' performance when cultivated in fertilized or non-fertilized substrate (Bretagnolle & Thompson, 2001). Nonetheless, the striking dearth of cultivation experiments comparing the performance of closely related diploid and tetraploid individuals (ideally from primary contact zones) under varying nutrient content does not yet allow for any generalization.

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AUTHOR CONTRIBUTION

F.K. and M.C. planned and designed the experiment. M.C., R.S. and J.S. collected plant material and soil at the serpentine locality, performed the experiment. M.C. and M.W. analyzed data, M.C., F.K. and M.W. wrote the manuscript.

REFERENCES

Baack EJ. **2004**. Cytotype segregation on regional and microgeographic scales in snow buttercups (*Ranunculus adoneus*: Ranunculaceae). *American Journal of Botany* **91**: 1783–1788.

Baack EJ. **2005**. To succeed globally, disperse locally: effects of local pollen and seed dispersal on tetraploid establishment. *Heredity* **94**: 538–546.

Baack EJ, Stanton ML. **2005**. Ecological factors influencing tetraploid speciation in snow buttercups (*Ranunculus adoneus*): Niche differentiation and tetraploid establishment. *Evolution* **59**: 1936–1944.

Barringer BC. **2007**. Polyploidy and self-fertilization in flowering plants. *American Journal of Botany* **94**: 1527–1533.

Brady KU, Kruckeberg AR, Bradshaw HD. **2005**. Evolutionary ecology of plant adaptation to serpentine soils. *Annual Review of Ecology, Evolution, and Systematics* **36**: 243–266.

Bretagnolle F, Lumaret R. **1995**. Bilateral polyploidization in *Dactylis glomerata* L . subsp. *lusitanica*: occurrence, morphological and genetic characteristics of first polyploids. *Euphytica* **84**: 197–207.

Bretagnolle F, Thompson JD. **2001**. Phenotypic plasticity in sympatric diploid and autotetraploid *Dactylis glomerata*. *International Journal of Plant Sciences* **162**: 309–316.

Collins AR, Naderi R, Mueller-Schaerer H. **2011**. Competition between cytotypes changes across a longitudinal gradient in *Centaurea stoebe* (Asteraceae). *American Journal of Botany* **98**: 1935–1942.

Doubková P, Suda J, Sudová R. **2012**. The symbiosis with arbuscular mycorrhizal fungi contributes to plant tolerance to serpentine edaphic stress. *Soil Biology and Biochemistry* **44**: 56–64.

Felber F. **1991**. Establishment of a tetraploid cytotype in a diploid population: Effect of relative fitness of the cytotypes. *Journal of Evolutionary Biology* **4**: 195–207.

Fowler NL, Levin DA. **1984**. Ecological constraints on the establishment of a novel polyploid in competition with its diploid progenitor. *The American Naturalist* **124**: 703–711.

Gaudet CL, Keddy PA. **1988**. A comparative approach to predicting competitive ability from plant traits. *Nature* **334**: 242–243.

Gauthier P, Lumaret R, Bédécarrats A. **1998**. Genetic variation and gene flow in Alpine diploid and tetraploid populations of *Lotus* (*L. alpinus* (D.C.) Schleicher/*L. corniculatus* L.). II. Insights from RFLP of chloroplast DNA. *Heredity* **80**: 694–701.

Grömping U. **2006**. Relative importance for linear regression in R: the package relaimpo. *Journal of Statistical Software* **17**: 1–27.

Guignard MS, Nichols RA, Knell RJ, Macdonald A, Romila C-A, Trimmer M, Leitch IJ, Leitch AR. **2016**. Genome size and ploidy influence angiosperm species' biomass under nitrogen and phosphorus limitation. *New Phytologist* **210**: 1195–1206.

Hanzl M, Kolář F, Nováková D, Suda J. **2014**. Nonadaptive processes governing early stages of polyploid evolution: Insights from a primary contact zone of relict serpentine *Knautia arvensis* (Caprifoliaceae). *American Journal of Botany* **101**: 935–945.

Hegarty M, Coate J, Sherman-Broyles S, Abbott R, Hiscock S, Doyle J. **2013**. Lessons from natural and artificial polyploids in higher plants. *Cytogenetic and Genome Research* **140**: 204–225.

Hothorn T, Bretz F, Westfall P. **2008**. Simultaneous inference in general parametric models. *Biometrical Journal* **50**: 346–363.

Husband BC. **2000**. Constraints on polyploid evolution: a test of the minority cytotype exclusion principle. *Proceedings of the Royal Society of London B* **267**: 217–223.

Husband BC. **2004**. The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. *Biological Journal of the Linnean Society* **82**: 537–546.

Husband BC, Ozimec B, Martin SL, Pollock L. **2008**. Mating consequences of polyploid evolution in flowering plants: Current trends and insights from synthetic polyploids. *International Journal of Plant Sciences* **169**: 195–206.

Chao D-Y, Dilkes B, Luo H, Douglas A, Yakubova E, Lahner B, Salt DE. **2013**. Polyploids exhibit higher potassium uptake and salinity tolerance in Arabidopsis. *Science* **341**: 658–9.

Jeník J. **1994**. Serpentine vegetation in Slavkov Forest, Western Bohemia. *Novitates botanicae Universitatis Carolinae* **8**: 51–62.

Kaplan Z. **1998**. Relict serpentine populations of *Knautia arvensis* s. l. (Dipsacaceae) in the Czech Republic and an adjacent area of Germany. *Preslia* **70:** 21–31.

Kazakou E, Dimitrakopoulos PG, Baker AJM, Reeves RD, Troumbis AY. **2008**. Hypotheses, mechanisms and trade-offs of tolerance and adaptation to serpentine soils: From species to ecosystem level. *Biological Reviews* **83**: 495–508.

Keddy P, Nielsen K, Weiher E, Lawson R. **2002**. Relative competitive performance of 63 species of terrestrial herbaceous plants. *Journal of Vegetation Science* **13**: 5–16.

Keeler KH, Davis GA. **1999**. Comparison of common cytotypes of *Andropogon gerardii* (Andropogoneae, Poaceae). *American Journal of Botany* **86**: 974–979.

Kennedy BF, Sabara HA, Haydon D, Husband BC. **2006**. Pollinator-mediated assortative mating in mixed ploidy populations of *Chamerion angustifolium* (Onagraceae). *Oecologia* **150**: 398–408.

Kim S, Rayburn AL, Boe A, Lee DK. **2012**. Neopolyploidy in *Spartina pectinata* Link: 1. Morphological analysis of tetraploid and hexaploid plants in a mixed natural population. *Plant Systematics and Evolution* **298**: 1073–1083.

Kolář F, Dortová M, Lepš J, Pouzar M, Krejčová A, Štech M. **2014**. Serpentine ecotypic differentiation in a polyploid plant complex: Shared tolerance to Mg and Ni stress among di- and tetraploid serpentine populations of *Knautia arvensis* (Dipsacaceae). *Plant Soil* **374**: 435–447.

Kolář F, Fér T, Štech M, Trávníček P, Dušková E, Schönswetter P, Suda J. **2012**. Bringing together evolution on serpentine and polyploidy: Spatiotemporal history of the diploid-tetraploid complex of *Knautia arvensis* (Dipsacaceae). *PLoS ONE* **7**: e39988.

Kolář F, Kaplan Z, Suda J, Štech M. **2015**. Populations of *Knautia* in ecologically distinct refugia on the Hercynian massif belong to two endemic species. *Preslia* **87**: 363–386.

Kolář F, Štech M, Trávníček P, Rauchová J, Urfus T, Vít P, Kubešová M, Suda J. **2009**. Towards resolving the *Knautia arvensis* agg. (Dipsacaceae) puzzle: Primary and secondary contact zones and ploidy segregation at landscape and microgeographic scales. *Annals of Botany* **103**: 963–974.

Levin DA. **1975**. Minority cytotype exclusion in local plant populations. *Taxon* **24**: 35–43.

Levin DA. **1983**. Polyploidy and novelty in flowering plants. *The American Naturalist* **122**: 1–25.

Levin DA. **2002**. *The role of chromosomal change in plant evolution*. Oxford: Oxford University Press.

Lindeman RH, Merenda PF, Gold RZ. **1980**. *Introduction to bivariate and multivariate analysis*. Glenview: Scott Foresman.

Lindsay WL, Norvell WA. **1978**. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal*: 421–428.

Lumaret R, Guillerm JL, Delay J, Ait Lhaj Loutfi A, Izco J, Jay M. **1987**. Polyploidy and habitat differentiation in *Dactylis glomerata* L. from Galicia (Spain). *Oecologia* **73**: 436–446.

Maceira NO, Jacquard P, Lumaret R. **1993**. Competition between diploid and derivative autotetraploid *Dactylis glomerata* L. from Galicia. Implications for the establishment of novel polyploid populations. *New Phytologist* **124**: 321–328.

Mehlich A. **1984**. Mehlich-3 soil test extractant: A modification of Mehlich-2 extractant. *Communications in Soil Science and Plant Analysis* **15**: 1409–1416.

Münzbergová Z. **2007**. No effect of ploidy level in plant response to competition in a common garden experiment. *Biological Journal of the Linnean Society* **92**: 211–219.

Nuismer SL, Cunningham BM. **2005**. Selection for phenotypic divergence between diploid and autotetraploid *Heuchera grossulariifolia*. *Evolution* **59**: 1928–1935.

Parisod C, Holderegger R, Brochmann C. **2010**. Evolutionary consequences of autopolyploidy. *New Phytologist*: 5–17.

Petit C, Bretagnolle F, Felber F. **1999**. Evolutionary consequences of diploid-polyploid hybrid zones in wild species. *Trends in Ecology and Evolution* **14**: 306–311.

Petit C, Lesbros P, Ge X, Thompson JD. **1997**. Variation in flowering phenology and selfing rate across a contact zone between diploid and tetraploid *Arrhenatherum elatius* (Poaceae). *Heredity* **79**: 31–40.

Proctor J, Woodell SRJ. **1975**. The ecology of serpentine soils. In: MacFadyen A [ed.] *Advances in ecological research*. Academic Press, 255–366.

R Core Team. **2014**. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramsey J. **2011**. Polyploidy and ecological adaptation in wild yarrow. *Proceedings of the National Academy of Sciences of the United States of America* **108**: 7096–7101.

Ramsey J, Ramsey TS. **2014**. Ecological studies of polyploidy in the 100 years following its discovery. *Philosophical Transactions of the Royal Society B* **369**: 20130352.

Ramsey J, Schemske DW. **1998**. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* **29**: 467–501.

Ramsey J, Schemske DW. **2002**. Neopolyploidy in Flowering Plants. *Annual Review of Ecology and Systematics* **33**: 589–639.

Rodríguez DJ. **1996**. A model for the establishment of polyploidy in plants. *The American Naturalist* **147**: 33–46.

Segraves KA, Thompson JN. **1999**. Plant polyploidy and pollination: Floral traits and insect visits to diploid and tetraploid *Heuchera grossulariifolia*. *Evolution* **53**: 1114–1127.

Soltis DE, Albert VA, Leebens-Mack J, Bell CD, Paterson AH, Zheng C, Sankoff D, DePamphilis CW, Wall PK, Soltis PS. **2009**. Polyploidy and angiosperm diversification. *American Journal of Botany* **96**: 336–348.

Soltis DE, Soltis PS. **1999**. Polyploidy: Recurrent formation and genome evolution. *Trends in Ecology and Evolution* **14**: 348–352.

Sonnleitner M, Flatscher R, Escobar García P, Rauchová J, Suda J, Schneeweiss GM, Hülber K, Schönswetter P. **2010**. Distribution and habitat segregation on different spatial scales among diploid, tetraploid and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps. *Annals of Botany* **106**: 967–977.

Stebbins GL. **1971**. *Chromosomal evolution in higher plants*. London: Edward Arnold.

Suda J, Herben T. **2013**. Ploidy frequencies in plants with ploidy heterogeneity: fitting a general gametic model to empirical population data. *Proceedings of the Royal Society of London B* **280**: 20122387.

Sudová R, Rydlová J, Münzbergová Z, Suda J. **2010**. Ploidy-specific interactions of three host plants with arbuscular mycorrhizal fungi: Does genome copy number matter? *American Journal of Botany* **97**: 1798–1807.

Šingliarová B, Chrtek J, Plačková I, Mráz P. **2011**. Allozyme variation in diploid, polyploid and mixed-ploidy populations of the *Pilosella alpicola* Group (Asteraceae): Relation to morphology, origin of polyploids and breeding system. *Folia Geobotanica* **46**: 387–410.

Šmarda P, Hejcman M, Březinová A, Horová L, Steigerová H, Zedek F, Bureš P, Hejcmanová P, Schellberg J. **2013**. Effect of phosphorus availability on the selection of species with different ploidy levels and genome sizes in a long-term grassland fertilization experiment. *New Phytologist* **200**: 911–921.

Štěpánek J. **1997**. *Knautia* L. In: Slavík B [ed]. *Květena České republiky*. Praha: Academia, 543–554.

Weiss-Schneeweiss H, Emadzade K, Jang TS, Schneeweiss GM. **2013**. Evolutionary consequences, constraints and potential of polyploidy in plants. *Cytogenetic and Genome Research* **140**: 137–150.

Wendel JF, Jackson SA, Meyers BC, Wing RA. **2016**. Evolution of plant genome architecture. *Genome Biology* **17**: 37.

Wood TE, Takebayashi N, Barker MS, Mayrose I, Greenspoon PB, Rieseberg LH. **2009**. The frequency of polyploid speciation in vascular plants. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 13875–13879.

Yamauchi A, Hosokawa A, Nagata H, Shimoda M. **2004**. Triploid bridge and role of parthenogenesis in the evolution of autopolyploidy. *The American Naturalist* **164**: 101–112.

Table S1 A list of progenitor diploid and derived tetraploid subpopulations of *Knautia serpentinicola* sampled at a serpentine locality in western Czechia where the two cytotypes coexist. The numbers of seeds subjected to experimental germination and seedlings entering subsequent cultivation are presented for each subpopulation of seed origin.

Subpopulation	Ploidy level	Coordinates (WGS-84)	Number of collected seeds	Number of cultivated plants
PV2D	2x	50.03774°N, 12.76646°E	383	62
VL105D	2x	50.03197°N, 12.74042°E	96	12
VL84D	2x	50.03218°N, 12.73805°E	197	36
PV33D	2x	50.03822°N, 12.76178°E	50	–
PV127T	4x	50.03869°N, 12.74456°E	330	38
PV12T	4x	50.03657°N, 12.76293°E	356	40
PV15T	4x	50.03777°N, 12.76390°E	185	32
PV19T	4x	50.03833°N, 12.76489°E	19	–

Table S2 A chemical analysis of the two substrate types used in experimental cultivation comparing their dry weight, pH, macronutrient concentrations and heavy-metal content. Soil samples were taken both before and after the experimental cultivation.

Substrate	D.w.[#]	pH_{KCl}	N	C_{total}	$C_{org.}$	Ca	Mg	K	Na	Ni	Co	Cr	Fe	P
	%		%	%	%	mg·kg^{-1}	mg·kg^{-1}	mg·kg^{-1}	mg·kg^{-1}	mg·kg^{-1}	mg·kg^{-1}	mg·kg^{-1}	mg·kg^{-1}	mg·kg^{-1}
Common garden$_{BC}$	98.2	7.2	0.32	7.47	7.44	4,590	330	369.2	40	0.7	0.15	0.15	26.9	193.1
Common garden$_{AC}$	97.5	7.3	0.35	7.74	7.69	4,770[*]	440	226.0	100[*]	0.7	0.09	0.19	62.8[*]	172.8
Native serpentine$_{BC}$	95.8	5.4	0.46	7.83	7.23	650	3,120	78.1	10	75.0	1.57	0.17	156.9	18.4
Native serpentine$_{AC}$	96.0	5.5	0.43	7.22	6.67	1,250[*]	2,730	51.7	160[*]	59.8	1.06	0.25	243.1[*]	5.2

BC = soil samples from before the cultivation, AC = soil samples from after the cultivation

[#] D.w. = Dry weight

* Increase in concentrations of these elements during the experimental cultivation, apparent in both common-garden and serpentine substrates, may have been caused by tap water used for regular automated irrigation in the greenhouse.

PAPER III

Evolutionary dynamics of mixed-ploidy populations in an annual herb: dispersal, local persistence and recurrent origins of polyploids

A fertile triploid individual of *Tripleurospermum inodorum* originating in a mixed-ploidy population among diploids and tetraploids.

Evolutionary dynamics of mixed-ploidy populations in an annual herb: dispersal, local persistence and recurrent origins of polyploids

Martin Čertner[1,2*], Eliška Fenclová[1], Pavel Kúr[1,3], Filip Kolář[1,2,4], Petr Koutecký[3], Anna Krahulcová[2] and Jan Suda[1,2]

[1]*Department of Botany, Faculty of Science, Charles University, Benátská 2, CZ-128 00 Prague, Czech Republic,* [2]*Institute of Botany, The Czech Academy of Sciences, Zámek 1, CZ-252 43 Průhonice, Czech Republic,* [3]*Department of Botany, Faculty of Science, University of South Bohemia, Branišovská 1760, CZ-370 05 České Budějovice, Czech Republic,* [4]*National Centre for Biosystematics, Natural History Museum, University of Oslo, NO-0318 Oslo, Norway*

**For correspondence. E-mail martin.certner@gmail.com*

Background and Aims Despite the recent wealth of studies targeted at contact zones of cytotypes in various species, some aspects of polyploid evolution are still poorly understood. This is especially the case for the frequency and success-rate of spontaneous neopolyploidization or the temporal dynamics of ploidy coexistence, requiring massive ploidy screening and repeated observations, respectively. To fill this gap, we initiated an extensive study of spatio-temporal patterns of ploidy coexistence in the widespread annual weed *Tripleurospermum inodorum* (Asteraceae).

Methods DNA flow cytometry along with confirmatory chromosome counts was employed to assess ploidy levels of 11,018 adult individuals and 1,263 *ex situ* germinated seedlings from 1,209 Central European populations. The ploidy screening was conducted across three spatial scales and supplemented with observations of temporal development of 37 mixed-ploidy populations.

Key Results The contact zone between the diploid and tetraploid cytotypes has a diffuse, mosaic-like structure enabling common cytotype coexistence from the within-population to the landscape level. A marked difference in monoploid genome size between the two cytotypes enabled the easy distinction of neotetraploid mutants from long-established tetraploids. Neotetraploids were extremely rare (0.03%) and occurred solitarily. Altogether five ploidy levels (2x-6x) and several aneuploids were discovered; the diversity in nuclear DNA content was highest in early ontogenetic stages (seedlings) and among individuals from mixed-ploidy populations. In spite of profound temporal oscillations in cytotype frequencies in mixed-ploidy populations, both diploids and tetraploids usually persisted up to the last census.

Conclusions Diploids and tetraploids commonly coexist at all spatial scales and exhibit considerable temporal stability in local ploidy mixtures. Mixed-ploidy populations containing fertile triploid hybrids likely act as effective generators of cytogenetic novelty and may facilitate inter-ploidy gene flow. Neopolyploid mutants were incapable of local establishment.

Key words: aneuploidy, annual plant, cytotype coexistence, flow cytometry, *Matricaria perforata*, mixed-ploidy population, neopolyploid, ploidy screening, temporal dynamics, *Tripleurospermum inodorum*, triploid

INTRODUCTION

Polyploidy (whole-genome duplication) is widely considered one of the most important evolutionary forces driving the diversification of flowering plants (Soltis *et al.*, 2009; Weiss-Schneeweiss *et al.*, 2013). It triggers both genotypic and phenotypic novelty (Levin, 1983; Flagel and Wendel, 2009; Hegarty *et al.*, 2013) and serves as an "instant" mechanism of sympatric speciation (Coyne and Orr, 2004; Wood *et al.*, 2009). Many plant species retain individuals of two or more different ploidy levels in certain parts of their distributional range (e.g. Kliber and Eckert, 2005; Castro *et al.*, 2012; Godsoe *et al.*, 2013; Kolář *et al.*, 2013) or even within their populations (e.g. Kao, 2007; Duchoslav *et al.*, 2010; Sonnleitner *et al.*, 2010; Trávníček *et al.*, 2011). Local coexistence of different cytotypes may arise either from an *in-situ* polyploidization event, resulting in the sympatric occurrence of genetically close di- and polyploids (i.e., primary contact) or through cytotype immigration into a population of another ploidy (i.e., secondary contact; Petit *et al.*, 1999). Mixed-ploidy populations serve as a convenient microcosm for the study of factors affecting the origin and establishment of polyploid derivatives (Husband, 2004; Hanzl *et al.*, 2014). In addition, they offer an opportunity to investigate the evolution of already established cytotypes that may be further subjected to inter-ploidy gene flow and transfer of adaptations (Chapman and Abbott, 2010; Arnold *et al.*, 2015; Hülber *et al.*, 2015).

According to theoretical predictions, the coexistence of two or more cytotypes within a population is unstable, representing only a transitory state before one of the cytotypes is locally fixed (Levin, 1975; Fowler and Levin, 1984). This stems from the fact that inter-ploidy mating results in progeny with reduced vitality, fertility or both (Ramsey and Schemske, 1998), making cytotype fitness dependent on frequency. The less abundant cytotype suffers more from the shortage of appropriate mates and is ultimately eliminated from the population (i.e., minority cytotype exclusion principle; Levin, 1975). Such a frequency-dependent mating disadvantage has been repeatedly observed both in experimental and natural mixed-ploidy populations (Hagberg and Ellerström, 1959; Husband, 2000; Baack, 2005a; Mráz *et al.*, 2012). However, there are several mechanisms that may compensate for the minority cytotype disadvantage (Rodríguez, 1996; Husband, 2004; Li *et al.*, 2004; Rausch and Morgan, 2005; Oswald and Nuismer, 2011). Firstly, it is only the outcome of sexual reproduction that is affected, while many species also rely on other modes of reproduction, such as vegetative propagation (Hroudová and Zákravský, 1993; Castro *et al.*, 2007) and apomixis (Kao, 2007; Mráz *et al.*, 2008). Secondly, the likelihood of a minority cytotype receiving compatible pollen increases through assortative (non-random) mating (Husband and Sabara, 2003). This can be achieved by shifts in flowering phenology of the cytotypes (Lumaret *et al.*, 1987; Petit *et al.*, 1997), different pollinator preferences (Segraves and Thompson, 1999; Kennedy *et al.*, 2006) or an increased rate of autogamy (Barringer, 2007). Assortative mating may also be promoted by spatial clustering of cytotypes (Baack, 2005b) as a result of ploidy-specific (micro-)habitat preferences (Lumaret *et al.*, 1987; Ramsey, 2011), human-mediated introduction of one cytotype into populations of another (Meirmans *et al.*, 1999; Mráz *et al.*, 2012; Otisková *et al.*, 2014) or a limited dispersal capacity (Baack, 2005b; Hanzl *et al.*, 2014). The persistence of a minority cytotype in mixed-ploidy populations can also be enhanced by its recurrent origins (Peckert and Chrtek, 2006; Ramsey, 2007), repeated immigration (Levin, 1975), superior competitive ability (Keeler and Davis, 1999) or better resistance to herbivore and parasite attacks (Nuismer and Thompson, 2001; Arvanitis *et al.*, 2008). Temporal oscillations in population size (resulting, for instance, from periodic disturbances) may also disrupt frequency-dependent selection and prevent the local fixation of one of the cytotypes (Rausch and Morgan, 2005; Halverson *et al.*, 2008).

Recent years have seen a considerable number of studies describing patterns in contact zones between different cytotypes of various species, examining processes allowing cytotype coexistence and improving our knowledge of the mechanisms of polyploid establishment (Husband and Sabara, 2003; Weiss-Schneeweiss *et al.*, 2013). However, we still know very little about the temporal aspect of ploidy coexistence, for which repeated observations are required, such as the fate of mixed-ploidy populations and the spatio-temporal dynamics of contact zones. The main challenge stems from the fact that the vast majority of heteroploid species subjected to detailed investigations are (long-lived) perennials (e.g. Lumaret *et al.*, 1987; Petit *et al.*, 1997; Keeler and Davis, 1999; Baack, 2005a; Kao, 2007; but see Buggs and Pannell, 2007; Manzaneda *et al.*, 2012), which precludes the assessment of their evolutionary dynamics in a reasonable time frame. Further understudied topics of polyploid evolution are rare evolutionary events such as the frequency of neopolyploid origins and the rate of successful polyploid establishment demanding massive ploidy screening both within and among populations (Halverson *et al.*, 2008). The relative contribution of recurrent polyploid origins to other processes stabilizing cytotype coexistence *in situ* is yet to be adequately addressed.

To fill these gaps, we focused on the spatio-temporal dynamics of ploidy coexistence in the common annual weed *Tripleurospermum inodorum* (Asteraceae). The species includes two morphologically indistinguishable cytotypes (Kay, 1969). Whereas diploids predominate in the western part of Europe, tetraploids are more common in its eastern parts, with a diffuse contact zone extending from France to Poland (Rottgardt, 1956; Kay, 1969; Lankosz-Mróz, 1976). Our pilot surveys revealed frequent coexistence of the two cytotypes in the Czech Republic, which provides a unique opportunity to study the diversity and distribution of cytotypes across several spatial scales – from within-population to landscape level. Using massive flow-cytometric screening across multiple life stages, spatial scales and years, we addressed the following questions: (1) What are the cytotype distribution patterns at various spatial scales and how common is ploidy coexistence? (2) Is there any consistent trend in the temporal development of local ploidy mixtures? (3) Is cytotype diversity at the seedling stage comparable with that in adult plants? (4) Given the differences in monoploid genome size between di- and tetraploids (see the Results), what is the frequency of spontaneous polyploid mutants in natural populations?

MATERIALS AND METHODS

Study species

Scentless mayweed [*Tripleurospermum inodorum* (L.) Sch. Bip., syn. *Matricaria perforata* Mérat, Asteraceae] is a common and widely distributed weed of arable land and other man-disturbed sites. Its native range covers most of Europe and Western Asia and it has been introduced to North America and some other temperate regions (Kay, 1976, 1994; Woo *et al.*, 1991).

Tripleurospermum inodorum is an annual herb with finely dissected tripinnate leaves and 0·2–0·7 m tall flowering stems terminated by one to a few hundred capitula (Kubát, 2004). The species is insect-pollinated and usually self-incompatible (Kay, 1965; Woo *et al.*, 1991); however, self-pollination in some populations has been reported (Kay, 1969). A typical plant produces thousands of achenes that lack any obvious adaptations for long-distance dispersal. Sexual reproduction is the only means of propagation, as the scentless mayweed is incapable of vegetative reproduction (Kay, 1994). Its achenes can survive for at least ten years buried in soil (Kay, 1994), which suggests that it develops a permanent soil seed bank (Bowes *et al.*, 1995). Germination and the formation of leaf rosettes takes place both in autumn and spring, resulting in a winter annual, spring annual or, less frequently, short-lived perennial life cycle (Woo *et al.*, 1991; Kubát, 2004).

Two morphologically indistinguishable cytotypes, diploid ($2n$ = 18) and tetraploid ($2n$ = 36), have been reported from both the native and the introduced ranges (Kay, 1969; Woo *et al.*, 1991). Diploids predominate in western (i.e., oceanic) parts of Europe whereas tetraploids prevail in more continental, eastern parts of the continent (Kay, 1969). A diffuse contact zone of both cytotypes extends from north-eastern France through Germany to Poland, but no mixed-ploidy populations have been reported (Rottgardt, 1956; Kay, 1969; Lankosz-Mróz, 1976). Interploidy breeding barriers seem to be weak, as artificial crosses of di- and tetraploids resulted in triploid seeds (Kay, 1965). An autopolyploid origin has been suggested for the tetraploid cytotype (Arora and Madhusoodanan, 1981).

Landscape, regional and local ploidy screening

Ploidy screening at the landscape level was conducted in Central Europe between 2011 and 2015, mainly focusing on the Czech Republic, where pilot data confirmed the occurrence of both diploids and tetraploids. The sampling was designed to cover regularly the study area and include various habitats. For each population, we recorded its position using a handheld GPS unit, briefly described the habitat and collected fresh leaves from at least ten randomly chosen individuals (whenever possible). Collected leaves were placed into plastic zip-lock bags and kept refrigerated until flow-cytometric analysis. Due to close resemblance between *T. inodorum* and *Matricaria* spp. at the stage of leaf rosettes, we attempted to sample unequivocally identifiable flowering individuals. If leaf rosettes were collected (<3% of populations), only samples that could be reliably identified based on their genome size were included.

To capture the structure of contact zones at a regional level, a detailed survey was carried out in three areas in the Czech Republic with common co-occurrence of di- and tetraploids: (i) in the surroundings of the town of Mariánské Lázně (MAR; 30 × 30 km; coordinates of the centroid 49°58'28"N, 12°45'29"E), (ii) in the surroundings of the town of Rakovník (RAK; 28 × 31 km; 50°09'59"N, 13°43'15"E) and (iii) E of the town of Soběslav (SOB; 21 × 23 km; 49°16'59"N, 14°52'13"E). The sampling strategy generally followed that of the landscape ploidy screening, but the sampling was much denser, and considerable effort was made to representatively cover all major habitat types.

The fine-scale distribution of di-, tetraploids and minority cytotypes was recorded in five mixed-ploidy populations (for more details, see Table 1). The size of each plot was adjusted to include >50 plants. The positions of all *T. inodorum* individuals were recorded, and one leaf per plant was collected for ploidy estimation.

Temporal dynamics of ploidy mixtures

We used two different approaches to describe temporal changes in ploidy composition of mixed-ploidy sites. At one of the fine-scale study plots (population no. 22), established in 2011, the distribution of cytotypes was recorded during the four consecutive years. Unfortunately, this approach for various reasons (e.g. low-abundant populations, sites on arable land unsuitable for the establishment of permanent plots, etc.) could not be applied to other mixed-ploidy sites.

As an alternative, we selected 36 mixed-ploidy populations discovered during the regional screening in 2011-2014 and resampled them in 2015. We only focused on populations where it was possible to repeat the sampling at exactly the same site and within approximately the same spatial bounds. Whenever possible, >20 plants per population were cytotyped in 2015.

Cytotype diversity of offspring

The ploidy screening of adult plants was complemented by analysis of *ex-situ* germinated seedlings. This approach enabled the capture of cytotype diversity at early ontogenetic stages of *T. inodorum* and thus to search for minority cytotypes with lower viability. Achenes were collected in one diploid, one tetraploid and five mixed-ploidy populations during autumn 2013. In each population, we pooled open-pollinated flower heads from >50 randomly chosen individuals, except for the two least abundant ones. The flower heads were stored in paper bags at room temperature until April 2014, when a few thousand achenes of each source population were sown in seedling trays filled with common garden soil. After three weeks, the ploidy level of at least 60 randomly selected seedlings per population was analysed, and only ploidy-variable populations were subjected to further sampling.

Due to extensive variability in DNA content of the offspring analysed, resulting in more-or-less continuous variation, we set arbitrary boundaries between the particular euploid cytotypes and aneuploids for a better overview of the results (Supplementary Data Fig. S1). Sixteen plants with known chromosome numbers were used as reference points. Aneuploid offspring was divided into three groups according to the nearest euploid ploidy levels: 2x–3x, 3x–4x, and 4x–5x.

Flow cytometry

Relative genome size of *T. inodorum* individuals was inferred from fluorescence intensities of DAPI-stained nuclei using flow cytometry (FCM). Sample preparation generally followed the two-step procedure using Otto buffers (Doležel *et al.*, 2007). Briefly, ca. 0·5 cm^2 of fresh sample leaf tissue and an appropriate amount of internal reference standard *Bellis perennis* L. (2C = 3·38 pg DNA; Schönswetter *et al.*, 2007) were chopped together in 0·5 mL of ice-cold extraction buffer (0·1 M citric acid, 0·5% Tween 20). The resulting suspension was filtered through a 42 μm nylon mesh, incubated for ca 5 min., and then mixed with 1 mL of a staining solution (0·4 M $Na_2HPO_4 \cdot 12H_2O$, 4 μg DAPI, 2 μL ß-mercaptoethanol). The fluorescence intensity of 3,000 particles was analysed using a Partec PA II flow cytometer (Partec GmbH, Münster, Germany) equipped with a UV LED chip. Up to ten individuals were pooled together during the ploidy screening, as our pilot analyses proved that minority cytotypes can be reliably detected even at low frequencies (<10%). Each plant was analysed separately when mixed-ploidy samples were encountered or if the quality of the resulting histograms was insufficient (i.e., coefficient of variation of the G_0/G_1 peak of the sample >4%). The interpretation of FCM results was based on chromosome counts obtained from reference plants in cultivation.

Chromosome counts

A subset of seedlings from mixed-ploidy populations representing the main categories of nuclear DNA content were transplanted into pots and cultivated in a greenhouse. Chromosomes were counted in somatic mitoses in root tips. Root tips were sampled in the morning, pretreated either with a saturated water solution of α-bromonaphtalene or with 0·002 M 8-hydroxyquinoline for 3·5–4 hours at room temperature, rinsed with water, fixed overnight in a cold mixture of ethanol and acetic acid (3:1) and stored in 70% ethanol at 4°C until required. The root tips were then hydrolysed in 1M HCl at 60°C for 7 min, rinsed with water, and their meristematic tissue was squashed in a drop of lacto-propionic orceine (Dyer, 1963). Chromosomes were counted in at least five metaphases per plant using an Olympus BX-51 microscope (total magnification 1,000×).

Data analysis

Differences in monoploid genome size (i.e. 1Cx values; Suda *et al.*, 2006) between diploids and tetraploids were compared using an analysis of variance (ANOVA) of 50 randomly selected flow-cytometric estimates for each of the cytotypes. Only single-individual analyses where the coefficient of variation of the *T. inodorum* sample was <3% were included.

Applied to the dataset of 36 mixed-ploidy populations revisited after 1–4 years, a Chi-square test was used to compare the numbers of populations turning uniformly diploid and uniformly tetraploid. The Kruskal-Wallis rank sum test was employed to test whether the time between the two sequential visits had a significant influence on population becoming ploidy-uniform or remaining mixed. An effect of habitat type on the fate of ploidy mixtures was tested using Fisher's exact test. Fisher's exact tests were also used for comparisons of cytotype composition (abundance of 2x, 3x, and 4x individuals) at each site between the two visits, and analogically, to evaluate between-year changes in cytotype composition of the mixed-ploidy population no. 22.

Spatial segregation of diploids and tetraploids within mixed-ploidy populations was assessed using the Mantel test (Mantel, 1967) implemented in the R package "ade4" (Dray and Dufour, 2007). A pairwise distance matrix derived from the position of individuals in each study plot was compared with a pairwise binary matrix coding cytotype identity. Triploids were omitted from spatial analyses due to their low abundance. Significance levels were estimated using a permutation test with 9,999 replicates.

RESULTS

Cytotype diversity and the origin of new tetraploid mutants

Flow-cytometric analyses of 11,018 individuals from 1,209 populations of *Tripleurospermum inodorum* yielded five distinct euploid cytotypes, ranging from the diploid to the hexaploid level (Fig. 1A). All ploidy levels with the exception of pentaploids were corroborated by chromosome counts: diploids ($2n = 2x = 18$; Fig. 1B), triploids ($2n = 3x = 27$; Fig. 1D), tetraploids ($2n = 4x = 36$; Fig. 1C) and hexaploids ($2n = 6x = 54$). Apart from diploids and tetraploids, triploids were the most common cytotype, occurring at a mean rate of 7·7% (s.e. = 0·95%) in mixed-ploidy populations. The other two minority cytotypes were extremely rare in natural populations, as only one pentaploid and one hexaploid were found (each constituting 0·01% of sampled individuals). The pentaploid grew in mixed-ploidy population no. 510 along with diploids and tetraploids, while the hexaploid

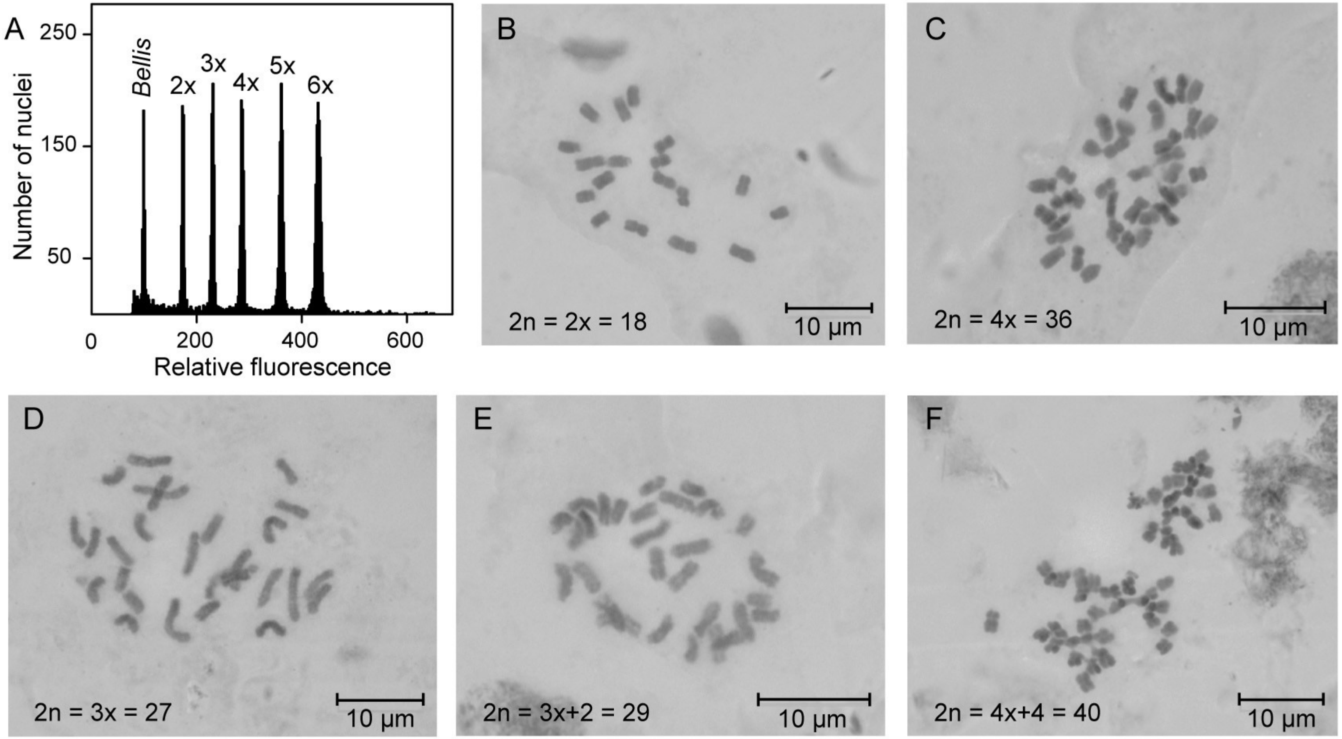

Figure 1. Flow-cytometric (FCM) estimates of relative genome size and confirmatory karyological analyses of *Tripleurospermum inodorum*. (A) Simultaneous FCM analysis of five individuals representing the five euploid cytotypes (2x–6x) distinguished during ploidy screening, with *Bellis perennis* as the internal standard. (B–D) Microphotographs of metaphase chromosomes of individuals belonging to the three most common ploidy levels: diploid (2*n* = 18; B), triploid (2*n* = 27; D), and tetraploid (2*n* = 36; C). (E,F) Metaphase chromosomes of two individuals identified as putative aneuploids in FCM analyses, and later confirmed as being hypertriploid (2*n* = 29; E) and hypertetraploid (2*n* = 40; F), respectively.

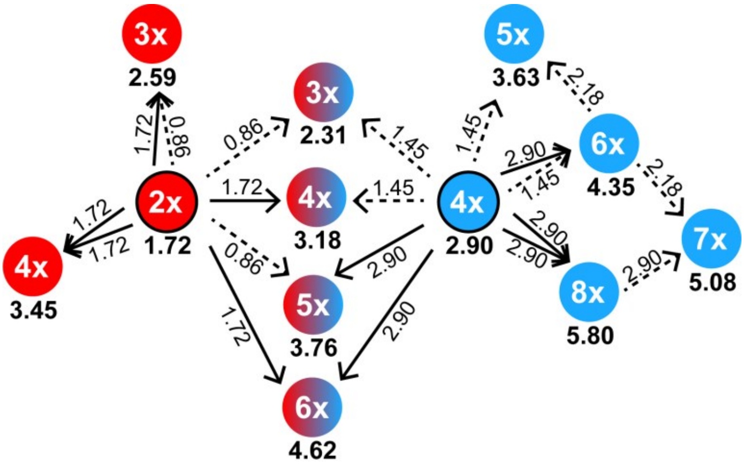

Figure 2. A theoretical overview of crosses involving reduced (dashed arrows) and unreduced (solid arrows) gametes of diploid (red with black stroke) and tetraploid (blue with black stroke) parents and cytotypes of the resulting offspring. Numbers refer to relative genome sizes of particular gametes (regular font) and cytotypes (bold font). Notice that some cytotypes have more than one way of origin and these can be easily distinguished based on relative genome size in *Tripleurospermum inodorum*. For simplicity, other possible crosses involving derived cytotypes are not shown.

occurred in the otherwise tetraploid population no. 1,057 (Supplementary Data Table S1). Interestingly, the relative genome size of tetraploids (2·90 ± 0·004 s.e., mean 1Cx = 0·726) did not equal double the diploid value (1·72 ± 0·008 s.e., mean 1Cx = 0·862; $F_{1, 98}$ = 2,242, $P << 0{\cdot}001$). The monoploid genome size of tetraploids was on average by ca 19% lower than that of diploids; the consistency of these differences was verified on selected individuals using propidium iodide flow cytometry (not shown). This lower monoploid genome size of established tetraploids enabled the easy detection of new polyploid mutants (i.e., neotetraploids) in routine flow-cytometric analyses (Fig. 2). Only three individuals with nuclear DNA content corresponding to neotetraploids (relative genome sizes of 3·26, 3·42, and 3·58) were identified in natural populations, constituting ca 0·03% of the samples analysed. These individuals were found singly in mixed-ploidy populations nos. 1,052 and 1,182, while the collection at site no. 187 consisted of one presumably neotetraploid plant from an unpaved field road, without any other *T. inodorum* individual in its proximity (Supplementary Data Table S1).

The relative genome size of triploids (2·28 ± 0·003 s.e.), intermediate between diploids and established tetraploids, is in accordance with their hybrid origin. Theoretically, the expected genome size of such hybrids is 2·31, contrary to the value of 2·59 expected for triploid mutants originating from a fusion of reduced and unreduced gametes of diploids (Fig. 2). On the other hand, the relative genome size of the single pentaploid individual (3·75) suggests its origin from a fusion of an unreduced gamete of an established tetraploid with a reduced gamete of a diploid (expected genome size = 3·76) rather than from hybridization between established tetraploid and hexaploid parents (expected genome size = 3·63). Aside from euploid cytotypes, five presumably aneuploid individuals were detected during our flow-cytometric ploidy screening, occurring singly in mixed-ploidy populations (nos. 189, 780, and 1,094) or in otherwise uniformly tetraploid populations (nos. 373, and 381; Supplementary Data Table S1). The nuclear DNA content of three of these putative aneuploids exceeded the tetraploid level whereas two other individuals were intermediary between diploids and triploids, and between triploids and tetraploids, respectively.

Spatial patterns of diploid – tetraploid coexistence

A total of 1,209 populations were screened for cytotype composition; most of them were purely tetraploid (823; 68%), mixed-ploidy populations were less frequent (271; 22%), and pure diploid populations were the rarest (115; 10%). While the predominant tetraploids occur in most of the sampled regions, diploids are much more scattered and presumably absent from some areas (e.g., the SE part of Central Europe and several regions within the Czech Republic; Fig. 3A). The contact zone of the two cytotypes has a diffuse, mosaic-like character.

More intensive ploidy screening was targeted at three regions within the Czech Republic (Figs. 3B-D). In W Bohemia (MAR; Fig. 3B), 358 populations were investigated, of which 184 (51·4%) were tetraploid, 120 (33·5%) mixed-ploidy and 54 (15·1%) diploid. Further 157 populations were screened in NW Bohemia (RAK; Fig. 3C), with 85 (54·1%) tetraploid, 52 (33·1%) mixed-ploidy and 20 (12·7%) diploid populations. Finally, 95 populations were visited in S Bohemia (SOB; Fig. 3D), consisting of 46 (48·4%) tetraploid, 41 (43·2%) mixed-ploidy and only 8 (8·4%) diploid populations. The cytotype distribution patterns are similar among all three regions: Tetraploid populations were the most common, and diploids were more frequently (2–5 times) found in mixed-ploidy populations than in pure diploid ones.

At the most detailed (intra-population) scale, the relative abundance of diploids and tetraploids varied across the sampled populations. Three were diploid-dominated whereas two exhibited similar frequencies of the two major cytotypes (Table 1). Triploid hybrids were discovered in four of these populations, in proportions ranging from 2·3% to 14·6%. Diploid and tetraploid individuals were more or less randomly scattered within the study plots (Figs. 3E, 4; Supplementary Data Fig. S2). Results of the Mantel test, however, suggested a slightly positive segregation of cytotypes in three of the five mixed-ploidy populations (Table 1).

Temporal changes in ploidy composition

Of the 36 mixed-ploidy populations in which temporal development of the cytotype composition was assessed after 1–4 years, 26 populations remained mixed-ploidy, four turned into purely diploid, and six became uniformly tetraploid.

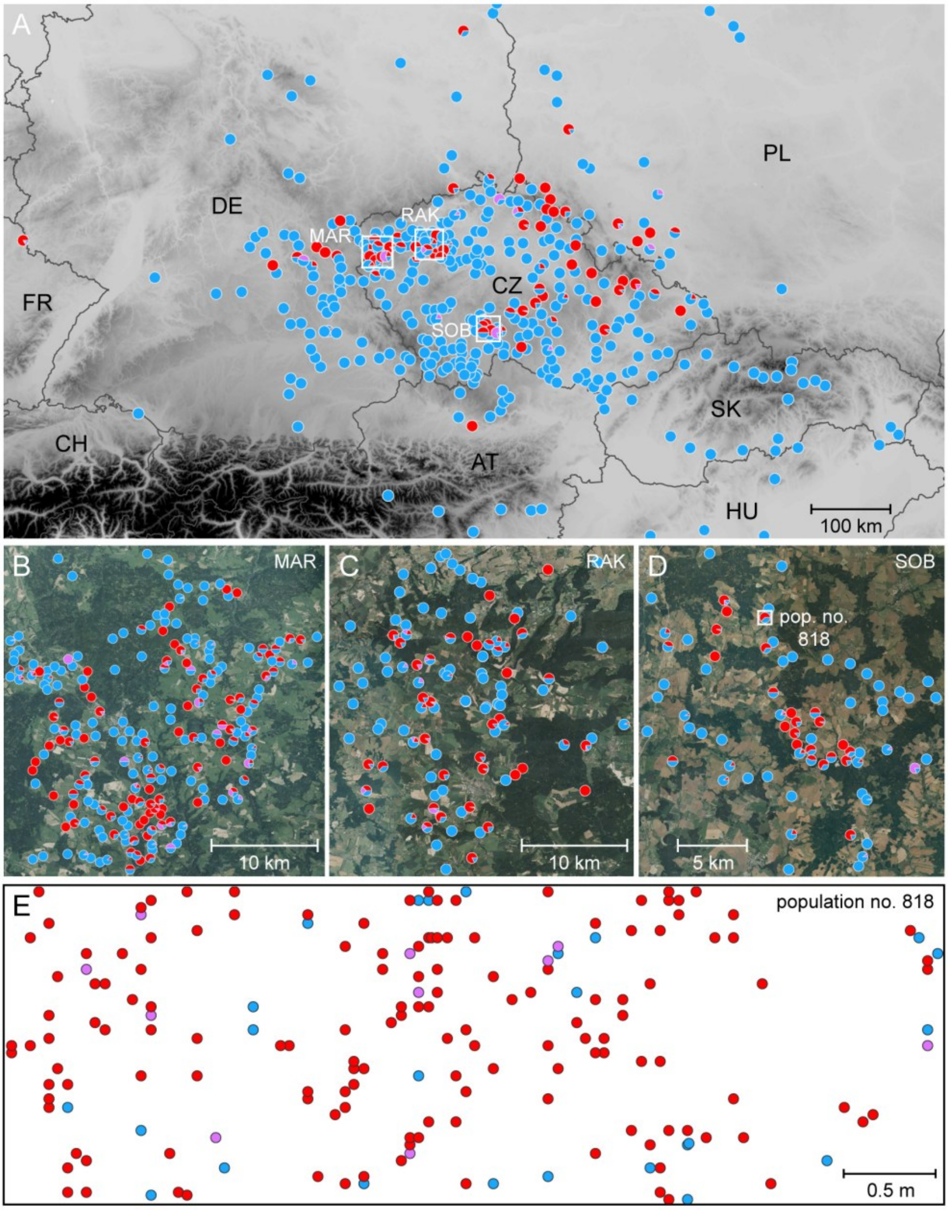

Figure 3. Structure of the contact zone between cytotypes of *Tripleurospermum inodorum* illustrating the coexistence of diploids (red), tetraploids (blue) and their triploid hybrids (violet) across three different spatial scales. Mixed-ploidy populations in (A–D) are presented as pie charts. (A) Cytotype distribution patterns in Central-European populations (11,018 analysed individuals). (B), (C) and (D) Three regions subjected to more detailed ploidy screening. (E) Small-scale distribution of di-, tri- and tetraploid individuals in one of the mixed-ploidy populations studied.

Table 1. Abundance and relative proportions of cytotypes and spatial segregation of diploids and tetraploids in five mixed-ploidy populations studied in detail.

Population[*]	Cytotype composition of population [count (ratio)]			Spatial segregation[†]	
	2x	3x	4x	r_M	P
818	144 (80.4%)	10 (5.6%)	25 (14.0%)	0.0568	0.060
22	60 (46.2%)	3 (2.3%)	67 (51.5%)	0.0907	**<0.001**
150	58 (75.3%)	3 (3.9%)	16 (20.8%)	-0.0293	0.803
916	23 (56.1%)	6 (14.6%)	12 (29.3%)	0.1289	**0.028**
889	40 (44.9%)	0 (0.0%)	49 (55.1%)	0.0479	**0.014**

[*] Population codes correspond to sequential numbers in the complete list of populations under study (Supplementary Data Table S1).

[†] Mantel correlation coefficient (r_M) and its significance level (P) showing spatial association of georeferenced diploid and tetraploid individuals (low-abundant triploids were omitted).

The two cytotypes did not differ in the probability of them prevailing in the initially mixed-ploidy population ($X^2 = 0{\cdot}4$, d.f. = 1, $P = 0{\cdot}527$), and the fate of ploidy mixtures did not depend on the time between the two sequential visits (Kruskal-Wallis test, $X^2 = 0{\cdot}209$, d.f. = 1, $P = 0{\cdot}648$) or the type of habitat (Fisher's exact test, $P = 0{\cdot}331$). However, significant temporal changes in ploidy composition were recorded only in ten of the 36 populations, four of them turned to be purely tetraploid and six remained mixed-ploidy (Table 2).

In one mixed-ploidy plot (population no. 22) that was re-collected yearly over five consecutive seasons, both diploids and tetraploids persisted until the last census in 2015. Interestingly, this was possible even despite a striking decrease in the overall abundance of the species and the fact that no plants were found at the locality in 2014 (Fig. 4). Between-year shifts in cytotype composition were significant only between 2012 and 2013 (Fisher's exact test, $P = 0{\cdot}007$).

Cytotype composition of offspring in mixed-ploidy populations

Flow-cytometric analyses of 1,263 seedlings yielded all five euploid cytotypes and a substantial number of putative aneuploids (Table 3). Although no minority cytotypes or aneuploids were identified among the progeny of one uniformly diploid population studied, a single hexaploid (1·6%) was detected in a uniformly tetraploid population. The greatest diversity in nuclear DNA content was found among offspring from mixed-ploidy populations. All these populations contained diploid, triploid and tetraploid offspring in various proportions, and three of them also included aneuploids (Table 3). Interestingly, the nuclear DNA content of the aneuploid offspring formed an almost perfect continuum spanning the 3x–4x and 4x–5x levels (Supplementary Data Fig. S1); note that a few of these putative aneuploids might be in fact triploid or tetraploid mutants originating from crosses involving unreduced gametes.

Table 2. Temporal shifts in ploidy composition at 36 initially mixed-ploidy sites sampled during our 2011-2014 field campaign and re-collected in 2015. Significant changes (α = 0.05) in cytotype abundance between the two visits are highlighted in bold.

Population*	Initial ploidy screening				Ploidy screening in 2015			Fisher's test	Habitat type
	season	2x	3x	4x	2x	3x	4x	*P*	
34	2012	6	–	1	18	–	–	0.280	field
547	2014	4	–	2	17	–	–	0.059	field
725	2014	10	–	3	20	–	–	0.052	field
665	2014	18	–	1	20	–	–	0.487	field
46	2012	**5**	**1**	**4**	**–**	**–**	**7**	**0.035**	ruderal site
323	2013	**3**	**4**	**19**	**–**	**–**	**44**	**<0.001**	pasture
313	2013	–	1	16	–	–	12	1	railway
900	2014	3	–	7	–	–	12	0.078	ruderal site
884	2014	**10**	**–**	**15**	**–**	**–**	**20**	**0.001**	field
932	2014	**8**	**3**	**8**	**–**	**–**	**20**	**<0.001**	field
83	2011	3	–	14	4	1	26	0.801	ruderal site
602	2012	8	–	2	22	1	–	0.085	ruderal site
181	2013	**3**	**3**	**1**	**25**	**2**	**16**	**0.021**	field
228	2013	17	2	–	17	–	2	0.237	field
226	2013	2	1	1	7	3	12	0.452	roadside
337	2013	**11**	**–**	**10**	**4**	**2**	**27**	**0.004**	field
215	2013	**11**	**1**	**25**	**17**	**–**	**10**	**0.016**	roadside
309	2013	–	1	8	1	–	15	0.600	field
293	2013	8	–	1	35	–	1	0.364	field
544	2014	4	–	7	14	1	17	0.800	forest drive
710	2014	3	1	10	11	1	18	0.537	fallow
556	2014	8	–	5	4	1	2	0.562	field
736	2014	**6**	**1**	**5**	**4**	**2**	**34**	**0.004**	field
802	2014	**5**	**1**	**5**	**1**	**–**	**14**	**0.011**	forest drive
471	2014	**9**	**1**	**3**	**9**	**3**	**18**	**0.037**	fallow
431	2014	7	2	1	14	3	13	0.106	field drive
671	2014	12	1	1	7	1	3	0.506	field
775	2014	2	3	12	1	1	18	0.383	field drive
778	2014	1	3	1	7	4	1	0.335	field drive
920	2014	12	–	2	19	–	1	0.555	field
1,095	2014	5	3	5	11	1	4	0.258	field
891	2014	18	1	1	26	2	2	1	field
889	2014	13	1	3	13	2	4	1	fallow
830	2014	4	–	16	4	1	17	1	roadside
819	2014	10	–	5	7	–	4	1	ruderal site
855	2014	9	–	6	4	–	4	0.685	field

*Population codes correspond to sequential numbers in the complete list of populations under study (Supplementary Data Table S1)

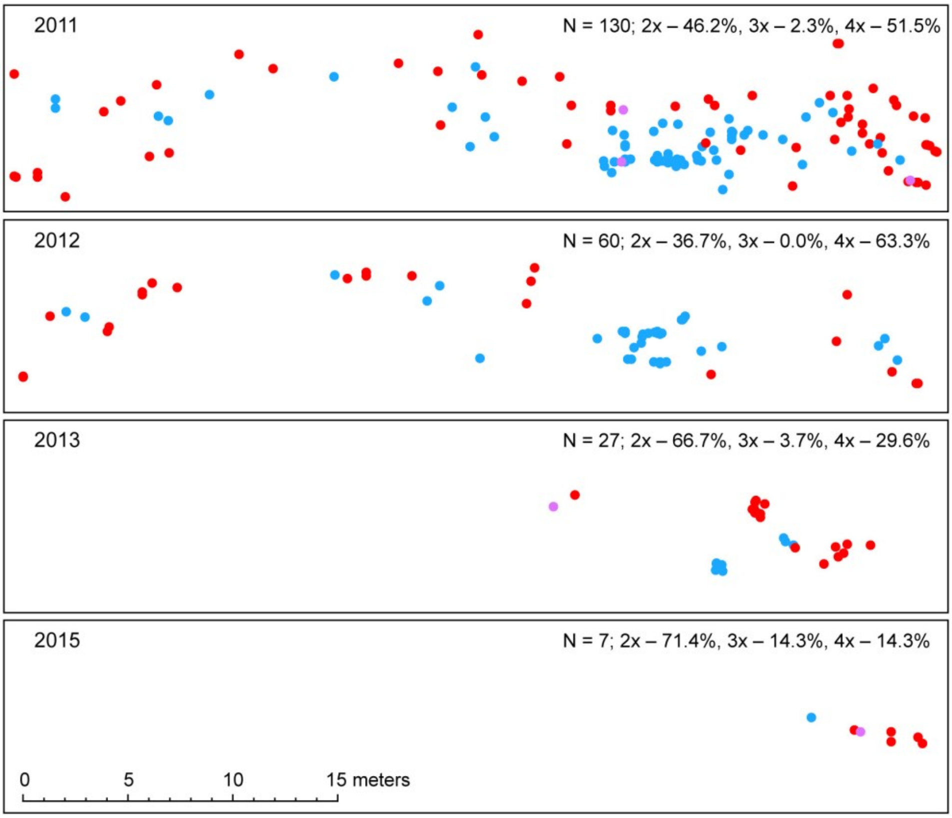

Figure 4. Spatio-temporal changes in the occurrence of diploids (red), triploids (violet) and tetraploids (blue) within a study plot set in the mixed-ploidy population no. 22. The plot was visited once a year from 2011 to 2015, and the position and ploidy level was assessed for all *Tripleurospermum inodorum* individuals within its bounds. No plants were found in 2014.

Five randomly selected aneuploid individuals were cultivated and subjected to chromosome counting, which revealed a hyper-triploid ($2n = 3x+2 = 29$; Fig. 1E) and four hyper-tetraploids ($2n = 4x+1\text{-}5 = 37\text{-}41$; Fig. 1F, Supplementary Data Table S2). The overall incidence of putatively aneuploid offspring ranged from 0% to 34% across the populations under study. Additionally, one pentaploid and three hexaploid individuals were identified in two of the mixed-ploidy populations (Table 3).

DISCUSSION

Cytotype coexistence at various spatial scales

Our extensive flow-cytometric ploidy screening documented a diffuse, mosaic-like contact zone of the diploid and tetraploid cytotypes of *T. inodorum* in Central Europe. It revealed that the two cytotypes commonly coexist at all spatial scales under study (Fig. 3). Though there are reports of diploids and tetraploids coexisting

Table 3: Diversity in nuclear DNA content among 1,263 seedlings germinated from pooled samples of open-pollinated achenes collected in five mixed-ploidy populations, one reference diploid population (no. 858), and one reference tetraploid population (no. 299). The offspring was assigned either to one of five euploid cytotypes (2x, 3x, 4x, 5x, and 6x), or to one of three arbitrarily-set classes of putative aneuploids (delimited by the closest lower and higher euploid cytotype: 2x–3x, 3x–4x, and 4x–5x).

Population*	Ploidy composition	Cytotypes at the level of seedlings [count (ratio)]								Total
		2x	2x–3x	3x	3x–4x	4x	4x–5x	5x	6x	
858	2x	76 (100.0%)	–	–	–	–	–	–	–	76
299	4x	–	–	–	–	62 (98.4%)	–	–	1 (1.6%)	63
66	2x, 3x, 4x	57 (32.4%)	–	9 (5.1%)	–	110 (62.5%)	–	–	–	176
46	2x, 3x, 4x	25 (9.6%)	–	37 (14.2%)	1 (0.4%)	197 (75.8%)	–	–	–	260
22	2x, 3x, 4x	98 (77.8%)	–	4 (3.2%)	–	24 (19.0%)	–	–	–	126
220	2x, 3x, 4x	7 (8.5%)	1 (1.2%)	34 (41.5%)	26 (31.7%)	12 (14.6%)	1 (1.2%)	1 (1.2%)	–	82
323	2x, 3x, 4x	18 (3.8%)	–	7 (1.5%)	4 (0.8%)	436 (90.8%)	12 (2.5%)	–	3 (0.6%)	480

*Population codes correspond to sequential numbers in the complete list of populations under study (Supplementary Data Table S1).

within the same regions in parts of Germany and Poland (Rottgardt, 1956; Kay, 1969; Lankosz-Mróz, 1976), mixed-ploidy populations have never been reported, and only the tetraploid cytotype had until now been known from the Czech Republic (Kubát, 2004). This discrepancy stems most likely from the much denser sampling in our study. Within three regions of the Czech Republic (medium-scale ploidy screening), mixed-ploidy populations were very common, reaching 33–43%. These values are greater than those documented in most other mixed-ploidy plant systems (e.g. McArthur and Sanderson, 1999; Baack, 2004; Stuessy *et al.*, 2004; Kolář *et al.*, 2009; Treier *et al.*, 2009; Duchoslav *et al.*, 2010; Castro *et al.*, 2012; Koutecký *et al.*, 2012; Manzaneda *et al.*, 2012). However, comparable or even higher frequencies of mixed-ploidy populations have been reported for some species such as *Andropogon gerardii* (McAllister *et al.*, 2015), *Arnica cordifolia* (Kao, 2007), *Galax urceolata* (Burton and Husband, 1999), *Gymnadenia conopsea* (Trávníček *et al.*, 2011), and *Senecio carniolicus* (Sonnleitner *et al.*, 2010).

At both the large and the medium scale, tetraploid populations of *T. inodorum* were clearly the most common in Central Europe. Very interesting is the striking shortage of uniformly diploid populations, which in all cases were 2–5 times less frequent than mixed-ploidy populations (composed of both diploids and tetraploids). The observed pattern of cytotype distribution could partly result from an interplay between migration and forces stabilizing cytotype coexistence in ploidy mixtures. Seed transfer is likely very common in this annual weed and may occasionally bring the two cytotypes together to form a mixed-ploidy population. Although the achenes of *T. inodorum* lack any obvious adaptations for long-distance dispersal, they can get effectively dispersed together with mud or soil adhering to the wheels of farm vehicles or the hooves of farm animals (Kay, 1994). Our study area is located at the eastern border of the Europe-wide contact zone, in tetraploid-dominated regions adjoining uniformly tetraploid parts of the *T. inodorum* range (Fig. 3A). Most immigrants are therefore likely to be tetraploid and many new populations will thus be founded by tetraploids. Tetraploid immigrants may also lead to conversion of diploid populations into ploidy mixtures and to the gradual fixation of the tetraploid cytotype in mixed-ploidy populations. Potentially, this scenario might lead to a gradual shift in cytotype composition at the landscape level in favour of tetraploids and thus result in the movement of the contact zone (*sensu* Buggs, 2007). The only well-documented case of a moving contact zone of cytotypes has been reported from Spanish populations of *Mercurialis annua*, where diploids were displacing hexaploids because of ploidy-specific differences in their sexual systems (Buggs and Pannell, 2006). In *T. inodorum*, persistent soil seed banks may slow the local turnover of the cytotypes driven by immigration, and theoretically, migration of diploids from the opposite direction would counteract the spread of the tetraploid. However, a long-term study of population dynamics at numerous sites (both ploidy-uniform and mixed) is necessary to address the potential instability of the contact zone in this species.

Nonetheless, there are also other factors that may influence the spatial structure of the contact zone. The two cytotypes may differ in their habitat requirements. If tetraploids have a wider ecological amplitude than diploids and the spectrum of habitats suitable for diploids mostly overlaps with that of tetraploids, then the number of uniformly diploid populations should indeed be much lower than the number of mixed or purely tetraploid populations. However, apart from a relationship with the macroclimate (Kay, 1969, 1994; Woo *et al.*, 1991), which is unlikely to shape the distribution of cytotypes at the medium-scale level (30 × 30 km in our case), we did not observe any differences between the cytotypes in a detailed vegetation survey (Čertner et al., in prep.). Finally, recurrent origins of tetraploids from diploids could have turned most diploid populations into mixtures of diploids and tetraploids. However, genome size data disprove the frequent formation of tetraploid mutants, as discussed below.

Rates of spontaneous polyploidization in natural populations

While the frequency of gametic non-reduction, as the main driver of polyploidization, is reasonably well understood (Bretagnolle and Thompson, 1995; Ramsey and Schemske, 1998; Levin, 2002; Sora *et al.*, 2016), the actual rate with which new polyploid mutants emerge and persist in natural populations is still mostly unknown (but see Ramsey, 2007). This rate can be approximated from the occurrence of higher-ploidy minority cytotypes in populations of their lower-ploid progenitors. However, certain cytotypes may originate both from crosses involving unreduced gametes in uniform-ploidy populations of progenitors and through inter-ploidy hybridization of established cytotypes (see Fig. 2), only the former pathway of cytotype formation being a reliable proxy of spontaneous polyploidization in natural populations.

In this respect, the profound 19% difference in monoploid genome sizes of diploids and tetraploids makes *T. inodorum* a unique study system. Given that the relative genome size of progeny is the sum of parental genome sizes (considering scenarios involving both reduced and unreduced gametes; Fig 2), we were able to distinguish between the alternative scenarios of polyploid formation and to confidently assess the rate of spontaneous polyploidization in populations of *T. inodorum*. In spite of our thorough sampling, polyploid mutants were extremely rare, as only one hexaploid and three neotetraploids were found among 11,018 adult individuals from 1,209 populations. The hexaploid originated in a tetraploid population by fusion of a reduced and an unreduced gamete. For the tetraploid mutants, two possible modes of origin were assumed (Fig. 2). Two individuals with relative genome sizes of 3·58 (population no. 187) and 3·42 (population no. 1,092) could have resulted from a fusion of two unreduced gametes of diploids (expected relative genome size = 3·45). A single plant with a relative genome size of 3·26 (population no. 1,227) might have originated from a fusion of an unreduced gamete of a diploid with a reduced gamete of a tetraploid (expected relative genome size =

3·18). Moreover, the overall rate of neotetraploid occurrence in natural populations may be even lower because the genome size of neotetraploids overlaps with that of certain aneuploids and we were unable to verify their status using chromosome counts.

The roughly similar incidence of neotetraploids and hexaploids suggests that the rates of spontaneous polyploidization in diploid and tetraploid parental populations are comparable. Neopolyploids always occurred solitarily, without any signs of their further spread, and were probably restricted to first generation. The mostly annual life cycle of *T. inodorum* along with the virtual lack of clonal propagation suggests that such neopolyploids are facing a strong minority disadvantage (Levin, 1975; Husband, 2000) and that their only chance of producing a new generation would be through autogamy. However, the self-compatibility of neopolyploids remains to be tested.

To our knowledge, the present study is the first to use pronounced differences in monoploid genome size of cytotypes to systematically screen for new polyploid mutants in diploid – polyploid contact zones. Generally, differences in monoploid genome sizes are indicators of independent evolution of particular lineages (Greilhuber, 2005), suggesting that tetraploids of *T. inodorum* either underwent substantial diversification following their origin or that they originated from a diploid lineage other than the one they are currently sympatric with. In both scenarios, the diploid – tetraploid contact zone of scentless mayweed represents an example of secondary cytotype contact (*sensu* Petit *et al.*, 1999).

Mixed-ploidy populations as generators of cytogenetic novelty

Our intensive screening of 1,263 seedlings from seven *T. inodorum* populations revealed not only all the five ploidy levels but also several aneuploids (spanning the 2x–3x, 3x–4x, and 4x–5x ploidy levels), thus capturing more cytotype diversity than was found among 11,018 adult individuals from across Central Europe. Specifically, aneuploids were more frequent among seedlings than among adult plants (4·0% vs. 0·3%, respectively; note that only individuals from mixed-ploidy populations are considered here), and more diverse in their relative genome size. Similarly, the incidence of hexaploid mutants was higher among seedlings (0·5% vs. 0·01%; relative to the overall number of tetraploids). Unfortunately, the more or less continuous variation in the genome size of analysed aneuploid seedlings (Supplementary Data Fig. S1) hindered such comparisons for triploid and tetraploid mutants.

Although the common use of pooled samples during our large- and medium-scale ploidy screening could have masked aneuploids differing by one or just a few chromosomes from the closest euploid level, the shortage of both aneuploids with more divergent genome sizes and neopolyploid mutants among adult plants is still evident. One possible explanation is that aneuploids and neopolyploids suffer from lower vitality and become outcompeted during later stages of the plant life cycle.

However, a subset of aneuploids and hexaploids grown under greenhouse conditions exhibited vigorous growth to maturity. We therefore attribute the deficiency of these cytotypes solely to the lower frequency of their emergence (Table 3). Given the massive seed production in *T. inodorum* (Kay, 1994) along with strong over-compensating density dependence (Buckley *et al.*, 2001), chance events are likely to prevent aneuploids and polyploid mutants from reaching maturity in many natural populations. The minority cytotype exclusion principle might further hinder their survival to subsequent generations (Levin, 1975) although it has no effect on cytotype diversity forming recurrently within each generation.

The degree of cytotype diversity at the seedling stage was tightly linked with the cytotype composition of populations. While only uniform-ploidy progeny was obtained by germinating seeds from both a uniformly diploid and a uniformly tetraploid population (with the exception of a single hexaploid individual in the tetraploid population), a minimum of three cytotypes were always present among progeny originating from mixed-ploidy populations and sometimes also aneuploids (Table 3). Aneuploid progeny most likely results from crosses in which either one or both parents are triploids. Triploids and other odd-level polyploids are known to produce gametes with a varying, unbalanced number of chromosomes (Ramsey and Schemske, 1998), giving rise to aneuploid progeny, as has been documented by experimental crosses (Krahulcová and Jarolímová, 1993; Norrmann *et al.*, 1997) and in natural mixed-ploidy populations (Keeler, 2004). Partial fertility of *T. inodorum* triploids was previously reported by Kay (1965), who observed triploids attaining a certain, albeit low, seed set when used as the egg parent in manipulated crosses with diploids or tetraploids. We also noticed triploid plants setting seed in natural mixed-ploidy populations during our field sampling.

Reproductive interactions between triploid hybrids and their parental cytotypes make mixed-ploidy populations of *T. inodorum* important generators of cytogenetic novelty. In a longer time frame, these processes might facilitate inter-ploidy gene flow between diploids and tetraploids (Comai, 2005), especially if also aneuploids are at least partly fertile. This would in turn strongly impact further evolution of the established cytotypes meeting in a secondary contact zone. Given the high frequency of mixed-ploidy populations and the considerable numbers of triploids within them (mean rate ca 8%), the common coexistence of *T. inodorum* cytotypes might have larger evolutionary significance, beyond a mere cytogeographical fact, and confer an adaptive advantage to the species. Manipulated pollinations and molecular-genetic analyses are currently in progress to study reproductive interactions among various euploid and aneuploid cytotypes of *T. inodorum* and their potential for mediating inter-ploidy gene flow.

Temporal stability of ploidy mixtures

The main constraints of local cytotype coexistence are inter-cytotype reproductive interactions (Levin, 1975; Husband, 2000; Li *et al.*, 2004; Rausch and

Morgan, 2005) and resource competition among cytotypes (Maceira *et al.*, 1993; Collins *et al.*, 2011). The strength of these mechanisms is generally influenced by specific life-traits of the given plant species. In *T. inodorum*, a lack of means of clonal reproduction (Kay, 1994) along with self-incompatibility reported from some populations (Kay, 1969) decrease reproductive assurance of a minority cytotype and thus the probability of cytotype coexistence. On the other hand, the spatial aggregation of plants of the same cytotype that we observed in three out of the five mixed-ploidy populations under study could increase non-random pollen transfer and restrict inter-cytotype competition (Baack, 2005b; Kennedy *et al.*, 2006). Given the lack of clonal growth, limited seed dispersal may be responsible for the spatial clumping of *T. inodorum* cytotypes. In addition, we have not noticed any striking ploidy-specific differences in plant size, vigour or reproductive effort (authors' personal observations) that could favour the persistence of one of the cytotypes. However, due to likely interactions between factors facilitating cytotype coexistence on the one hand, and factors leading to the fixation of one of the cytotypes on the other, the most reliable way of assessing the fate of ploidy mixtures are temporal observations of their development.

Despite our initial expectations of rapid fixation of one of the cytotypes in mixed-ploidy populations of this annual weed, our temporal observations suggest surprisingly high stability of ploidy coexistence. In one mixed-ploidy population screened annually over a five-year period, both diploids and tetraploids persisted at the site until the last season, in spite of a severe gradual reduction of population size and even an absence of adults and seedlings in one year (Fig. 4). Similarly, most of the 36 mixed-ploidy sites re-analysed for ploidy composition (72%) contained both diploids and tetraploids after 1–4 years (Table 2). It is likely that permanent soil seed banks were established at these sites in the first year of our study or even earlier. Subsequent germination of variably aged seeds made the cytotype composition of populations in each given year more or less independent of the year before. The effect of permanent soil seed banks is often neglected in polyploid studies (but see Hahn et al. 2012), but it may play an important role in stabilizing mixed-ploidy populations, especially of annual and short-lived plants. Moreover, repeated immigration of seeds of particular cytotypes from surrounding populations may have a similar effect. Finally, the fixation of one of the cytotypes in mixed-ploidy populations might be slowed down or even hampered by disturbances that prevent the dominant cytotype from attaining the highest population densities (Halverson *et al.*, 2008).

To our knowledge, this study is the first to document temporal changes in cytotype composition *in situ*, at the within-population scale. We are aware of the relatively short observation periods in our study, which precludes reaching more general conclusions. Also, the fact that our tests showed no effect of habitat and/or time between the two sequential visits on the fate of ploidy mixtures could be partially attributed to their low statistical power. More comprehensive assessments of temporal changes in cytotype composition of mixed-ploidy sites are currently being undertaken, along with a targeted study of potentially stabilizing mechanisms.

CONCLUSIONS

Extensive flow-cytometric screening of over 11,000 adult individuals from 1,209 populations supplemented with more than 1,200 *ex situ* germinated seedlings was employed to survey a diploid – tetraploid contact zone of *T. inodorum* in Central Europe. The contact zone has a diffuse, mosaic-like structure enabling the common coexistence of the two cytotypes at all spatial scales studied. Mixed-ploidy populations, containing both diploids and tetraploids, then likely act as effective generators of cytogenetic novelty. This was especially apparent from the striking variation in DNA content of offspring from mixed-ploidy sites, which could be attributed to ongoing reproductive interactions between relatively numerous triploid hybrids and their parental cytotypes. In a longer time frame, such reproductive interactions might even facilitate inter-ploidy gene flow from diploids to tetraploids and *vice versa*. The local coexistence of diploids and long-established tetraploids meeting in certain parts of their overlapping distributional range might thus leave significant traces in the cytotypes' evolutionary history or even confer an adaptive advantage to the species (e.g. through the transfer of adaptations).

Our study species has two unique aspects when compared with other well-documented mixed-ploidy systems. Firstly, the profound 19% difference in monoploid genome size between diploids and tetraploids enables the easy distinction of neotetraploid mutants from long-established tetraploids during routine flow-cytometric ploidy screening. Neotetraploid mutants were extremely rare among adult individuals of *T. inodorum* (approximately 0·03%); they never formed uniform populations but occurred solitarily, without any sign of their local establishment and further spread. Secondly, aside from capturing the spatial distribution of ploidy diversity, the predominantly annual life-cycle of the species also allows the monitoring of trends in the temporal development of this diversity. In spite of considerable oscillations in cytotype frequencies and the overall abundance of mixed-ploidy populations, our observations suggest that the local coexistence of diploids and tetraploids is surprisingly stable. The persistence of mixed-ploidy populations is likely driven by a combined effect of permanent soil seed banks, repeated immigration of seeds of particular cytotypes from surrounding populations and recurring local disturbances.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: the complete list of populations sampled in our study. Table S2: relative genome size and chromosome counts of *ex-situ* germinated seedlings. Figure S1: distribution of relative genome size among seedlings and arbitrary delimitation of euploid and aneuploid cytotypes. Figure S2: small-scale distribution of cytotypes in three mixed-ploidy populations.

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LITERATURE CITED

Arnold B, Kim ST, Bomblies K. 2015. Single geographic origin of a widespread autotetraploid *Arabidopsis arenosa* lineage followed by interploidy admixture. *Molecular Biology and Evolution* **32**: 1382–1395.

Arora OP, Madhusoodanan KJ. 1981. Nature of tetraploidy in *Matricaria inodora* L. *Cytologia* **46**: 773–779.

Arvanitis L, Wiklund C, Ehrlén J. 2008. Plant ploidy level influences selection by butterfly seed predators. *Oikos* **117**: 1020–1025.

Baack EJ. 2004. Cytotype segregation on regional and microgeographic scales in snow buttercups (*Ranunculus adoneus*: Ranunculaceae). *American Journal of Botany* **91**: 1783–1788.

Baack EJ. 2005a. Ecological factors influencing tetraploid establishment in snow buttercups (*Ranunculus adoneus*, Ranunculaceae): Minority cytotype exclusion and barriers to triploid formation. *American Journal of Botany* **92**: 1827–1835.

Baack EJ. 2005b. To succeed globally, disperse locally: effects of local pollen and seed dispersal on tetraploid establishment. *Heredity* **94**: 538–546.

Barringer BC. 2007. Polyploidy and self-fertilization in flowering plants. *American Journal of Botany* **94**: 1527–1533.

Bowes GG, Thomas AG, Lefkovitch LP. 1995. Changes with time in the germination of buried scentless chamomile (*Matricaria perforata* Mérat) seeds. *Canadian Journal of Plant Science* **75**: 277–281.

Bretagnolle F, Thompson JD. 1995. Tansley review no. 78. Gametes with the somatic chromosome number: mechanisms of their formation and role in the evolution of autopolypoid plants. *New Phytologist* **129**: 1–22.

Buckley YM, Hinz HL, Matthies D, Rees M. 2001. Interactions between density-dependent processes, population dynamics and control of an invasive plant species, *Tripleurospermum perforatum* (scentless chamomile). *Ecology Letters* **4**: 551–558.

Buggs RJA. 2007. Empirical study of hybrid zone movement. *Heredity* **99**: 301–312.

Buggs RJA, Pannell JR. 2006. Rapid displacement of a monoecious plant lineage is due to pollen swamping by a dioecious relative. *Current Biology* **16**: 996–1000.

Buggs RJA, Pannell JR. 2007. Ecological differentiation and diploid superiority across a moving ploidy contact zone. *Evolution* **61**: 125–140.

Burton TL, Husband BC. 1999. Population cytotype structure in the polyploid *Galax urceolata* (Diapensiaceae). *Heredity* **82**: 381–390.

Castro S, Loureiro J, Procházka T, Münzbergová Z. 2012. Cytotype distribution at a diploid-hexaploid contact zone in *Aster amellus* (Asteraceae). *Annals of Botany* **110**: 1047–1055.

Castro S, Loureiro J, Santos C, Ater M, Ayensa G, Navarro L. 2007. Distribution of flower morphs, ploidy level and sexual reproduction of the invasive weed *Oxalis pes-caprae* in the western area of the Mediterranean region. *Annals of Botany* **99**: 507–517.

Chapman MA, Abbott RJ. 2010. Introgression of fitness genes across a ploidy barrier. *New Phytologist* **186**: 63–71.

Collins AR, Naderi R, Mueller-Schaerer H. 2011. Competition between cytotypes changes across a longitudinal gradient in *Centaurea stoebe* (Asteraceae). *American Journal of Botany* **98**: 1935–1942.

Comai L. 2005. The advantages and disadvantages of being polyploid. *Nature Reviews. Genetics*. **6:** 836–846.

Coyne JA, Orr HA. 2004. *Speciation*. Sunderland: Sinauer Associates.

Doležel J, Greilhuber J, Suda J. 2007. Estimation of nuclear DNA content in plants using flow cytometry. *Nature protocols* **2**: 2233–2244.

Dray S, Dufour AB. 2007. The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software* **22**: 1–20.

Duchoslav M, Šafářová L, Krahulec F. 2010. Complex distribution patterns, ecology and coexistence of ploidy levels of *Allium oleraceum* (Alliaceae) in the Czech Republic. *Annals of Botany* **105**: 719–735.

Dyer AF. 1963. The use of lacto-propionic orcein in rapid squash methods for chromosome preparations. *Stain Technology* **38**: 85–90.

Flagel LE, Wendel JF. 2009. Gene duplication and evolutionary novelty in plants. *New Phytologist* **183**: 557–564.

Fowler NL, Levin DA. 1984. Ecological constraints on the establishment of a novel polyploid in competition with its diploid progenitor. *The American Naturalist* **124**: 703–711.

Godsoe W, Larson MA, Glennon KL, Segraves KA. 2013. Polyploidization in *Heuchera cylindrica* (Saxifragaceae) did not result in a shift in climatic requirements. *American Journal of Botany* **100**: 496–508.

Greilhuber J. 2005. Intraspecific variation in genome size in angiosperms: identifying its existence. *Annals of Botany* **95:** 91–98.

Hagberg A, Ellerström S. 1959. The competition between diploid, tetraploid and aneuploid rye: theoretical and practical aspects. *Hereditas* **45**: 369–416.

Hahn MA, Buckley YM, Müller-Schärer H. 2012. Increased population growth rate in invasive polyploid *Centaurea stoebe* in a common garden. *Ecology Letters* **15:** 947–954.

Halverson K, Heard SB, Nason JD, Stireman JO. 2008. Origins, distribution, and local co-occurrence of polyploid cytotypes in *Solidago altissima* (Asteraceae). *American Journal of Botany* **95**: 50–58.

Hanzl M, Kolář F, Nováková D, Suda J. 2014. Nonadaptive processes governing early stages of polyploid evolution: insights from a primary contact zone of relict serpentine *Knautia arvensis* (Caprifoliaceae). *American Journal of Botany* **101**: 935–945.

Hegarty M, Coate J, Sherman-Broyles S, Abbott R, Hiscock S, Doyle J. 2013. Lessons from natural and artificial polyploids in higher plants. *Cytogenetic and Genome Research* **140**: 204–225.

Hroudová Z, Zákravský P. 1993. Ecology of two cytotypes of *Butomus umbellatus* II: reproduction, growth and biomass production. *Folia Geobotanica & Phytotaxonomica* **28**: 413–424.

Hülber K, Sonnleitner M, Suda J *et al.* 2015. Ecological differentiation, lack of hybrids involving diploids, and asymmetric gene flow between polyploids in narrow contact zones of *Senecio carniolicus* (syn. *Jacobaea carniolica*, Asteraceae). *Ecology and Evolution* **5**: 1224–1234.

Husband BC. 2000. Constraints on polyploid evolution: a test of the minority cytotype exclusion principle. *Proceedings of the Royal Society of London, Series B – Biological Sciences* **267**: 217–223.

Husband BC. 2004. The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. *Biological Journal of the Linnean Society* **82**: 537–546.

Husband BC, Sabara HA. 2003. Reproductive isolation between autotetraploids and their diploid progenitors in fireweed, *Chamerion angustifolium* (Onagraceae). *New Phytologist* **161**: 703–713.

Kao RH. 2007. Asexuality and the coexistence of cytotypes. *New Phytologist* **175**: 764–772.

Kay QON. 1965. *Experimental and comparative ecological studies of selected weeds*. PhD Thesis, University of Oxford, UK.

Kay QON. 1969. The origin and distribution of diploid and tetraploid *Tripleurospermum inodorum* (L.) Schultz Bip. *Watsonia* **7**: 130–141.

Kay QON. 1976. 60. *Matricaria* L. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA, eds. *Flora Europaea, Vol. 4*. Cambridge: Cambridge University Press, 165–167.

Kay QON. 1994. *Tripleurospermum inodorum* (L.) Schultz Bip. *Journal of Ecology* **82**: 681–697.

Keeler KH. 2004. Impact of intraspecific polyploidy in *Andropogon gerardii* (Poaceae) populations. *The American Midland Naturalist* **152**: 63–74.

Keeler KH, Davis GA. 1999. Comparison of common cytotypes of *Andropogon gerardii* (Andropogoneae, Poaceae). *American Journal of Botany* **86**: 974–979.

Kennedy BF, Sabara HA, Haydon D, Husband BC. 2006. Pollinator-mediated assortative mating in mixed ploidy populations of *Chamerion angustifolium* (Onagraceae). *Oecologia* **150**: 398–408.

Kliber A, Eckert CG. 2005. Interaction between founder effect and selection during biological invasion in an aquatic plant. *Evolution* **59**: 1900–1913.

Kolář F, Lučanová M, Vít P *et al.* 2013. Diversity and endemism in deglaciated areas: ploidy, relative genome size and niche differentiation in the *Galium pusillum* complex (Rubiaceae) in Northern and Central Europe. *Annals of Botany* **111**: 1095–1108.

Kolář F, Štech M, Trávníček P *et al.* 2009. Towards resolving the *Knautia arvensis* agg. (Dipsacaceae) puzzle: primary and secondary contact zones and ploidy segregation at landscape and microgeographic scales. *Annals of Botany* **103**: 963–974.

Koutecký P, Štěpánek J, Baďurová T. 2012. Differentiation between diploid and tetraploid *Centaurea phrygia*: mating barriers, morphology and geographic distribution. *Preslia* **84**: 1–32.

Krahulcová A, Jarolímová V. 1993. Ecology of two cytotypes of *Butomus umbellatus* I. Karyology and breeding behaviour. *Folia Geobotanica & Phytotaxonomica* **28**: 385–411.

Kubát K. 2004. 33. *Tripleurospermum* Schultz Bip. - heřmánkovec. In: Slavík B, Štěpánková J, eds. *Květena České republiky, Vol. 7*. Praha: Academia, 248–250.

Lankosz-Mróz M. 1976. Karyological investigations on *Tripleurospermum maritimum* (L.) Koch ssp. *inodorum* (L.) ex Vaarama from Poland. *Acta Biologica Cracoviensia (Series Botanica)* **19**: 93–105.

Levin DA. 1975. Minority cytotype exclusion in local plant populations. *Taxon* **24**: 35–43.

Levin DA. 1983. Polyploidy and novelty in flowering plants. *The American Naturalist* **122**: 1–25.

Levin DA. 2002. *The role of chromosomal change in plant evolution*. Oxford: Oxford University Press.

Li BH, Xu XM, Ridout MS. 2004. Modelling the establishment and spread of autotetraploid plants in a spatially heterogeneous environment. *Journal of Evolutionary Biology* **17**: 562–573.

Lumaret R, Guillerm JL, Delay J, Ait Lhaj Loutfi A, Izco J, Jay M. 1987. Polyploidy and habitat differentiation in *Dactylis glomerata* L. from Galicia (Spain). *Oecologia* **73**: 436–446.

Maceira NO, Jacquard P, Lumaret R. 1993. Competition between diploid and derivative autotetraploid *Dactylis glomerata* L. from Galicia. Implications for the establishment of novel polyploid populations. *New Phytologist* **124**: 321–328.

Mantel N. 1967. The detection of disease clustering and a general regression approach. *Cancer Research* **27**: 209–220.

Manzaneda AJ, Rey PJ, Bastida JM, Weiss-Lehman C, Raskin E, Mitchell-Olds T. 2012. Environmental aridity is associated with cytotype segregation and polyploidy occurrence in *Brachypodium distachyon* (Poaceae). *The New Phytologist* **193**: 797–805.

McAllister C, Blaine R, Kron P *et al.* 2015. Environmental correlates of cytotype distribution in *Andropogon gerardii* (Poaceae). *American Journal of Botany* **102**: 92–102.

McArthur ED, Sanderson SC. 1999. Cytogeography and chromosome evolution of subgenus Tridentatae of *Artemisia* (Asteraceae). *American Journal of Botany* **86**: 1754–1775.

Meirmans PG, Calame FG, Bretagnolle F, Felber F, den Nijs JCM. 1999. Anthropogenic disturbance and habitat differentiation between sexual diploid and apomictic triploid *Taraxacum* sect. Ruderalia. *Folia Geobotanica* **34**: 451–469.

Mráz P, Šingliarová B, Urfus T, Krahulec F. 2008. Cytogeography of *Pilosella officinarum* (Compositae): altitudinal and longitudinal differences in ploidy level distribution in the Czech Republic and Slovakia and the general pattern in Europe. *Annals of Botany* **101**: 59–71.

Mráz P, Španiel S, Keller A *et al.* 2012. Anthropogenic disturbance as a driver of microspatial and microhabitat segregation of cytotypes of *Centaurea stoebe* and cytotype interactions in secondary contact zones. *Annals of Botany* **110**: 615–627.

Norrmann GA, Quarín CL, Keeler KH. 1997. Evolutionary implications of meiotic chromosome behavior, reproductive biology, and hybridization in 6x and 9x cytotypes of *Andropogon gerardii* (Poaceae). *American Journal of Botany* **84**: 201–207.

Nuismer SL, Thompson JN. 2001. Plant polyploidy and non-uniform effects on insect herbivores. *Proceedings of the Royal Society of London, Series B – Biological Sciences* **268**: 1937–1940.

Oswald BP, Nuismer SL. 2011. Neopolyploidy and diversification in *Heuchera grossulariifolia*. *Evolution* **65**: 1667–1679.

Otisková V, Koutecký T, Kolář F, Koutecký P. 2014. Occurrence and habitat preferences of diploid and tetraploid cytotypes of *Centaurea stoebe* in the Czech Republic. *Preslia* **86**: 67–80.

Peckert T, Chrtek J. 2006. Mating interactions between coexisting diploid, triploid and tetraploid cytotypes of *Hieracium echioides* (Asteraceae). *Folia Geobotanica* **41**: 323–334.

Petit C, Bretagnolle F, Felber F. 1999. Evolutionary consequences of diploid-polyploid hybrid zones in wild species. *Trends in Ecology and Evolution* **14**: 306–311.

Petit C, Lesbros P, Ge X, Thompson JD. 1997. Variation in flowering phenology and selfing rate across a contact zone between diploid and tetraploid *Arrhenatherum elatius* (Poaceae). *Heredity* **79**: 31–40.

Ramsey J. 2007. Unreduced gametes and neopolyploids in natural populations of *Achillea borealis* (Asteraceae). *Heredity* **98**: 143–150.

Ramsey J. 2011. Polyploidy and ecological adaptation in wild yarrow. *Proceedings of the National Academy of Sciences of the United States of America* **108**: 7096–7101.

Ramsey J, Schemske DW. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* **29**: 467–501.

Rausch JH, Morgan MT. 2005. The effect of self-fertilization, inbreeding depression, and population size on autopolyploid establishment. *Evolution* **59**: 1867–1875.

Rodríguez DJ. 1996. A model for the establishment of polyploidy in plants. *The American Naturalist* **147**: 33–46.

Rottgardt K. 1956. Morphologische, cytologische und physiologische Untersuchungen von Ökotypen in Schleswig-Holstein. *Beiträge zur Biologie der Pflanzen* **32**: 225–278.

Segraves KA, Thompson JN. 1999. Plant polyploidy and pollination: floral traits and insect visits to diploid and tetraploid *Heuchera grossulariifolia*. *Evolution* **53**: 1114–1127.

Schönswetter P, Suda J, Popp M, Weiss-Schneeweiss H, Brochmann C. 2007. Circumpolar phylogeography of *Juncus biglumis* (Juncaceae) inferred from AFLP fingerprints, cpDNA sequences, nuclear DNA content and chromosome numbers. *Molecular Phylogenetics and Evolution* **42**: 92–103.

Soltis DE, Albert VA, Leebens-Mack J *et al.* 2009. Polyploidy and angiosperm diversification. *American Journal of Botany* **96**: 336–348.

Sonnleitner M, Flatscher R, Escobar García P *et al.* 2010. Distribution and habitat segregation on different spatial scales among diploid, tetraploid and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps. *Annals of Botany* **106**: 967–77.

Sora D, Kron P, Husband BC. 2016. Genetic and environmental determinants of unreduced gamete production in *Brassica napus*, *Sinapis arvensis* and their hybrids. *Heredity* **117**: 440–448.

Stuessy TF, Weiss-Schneeweiss H, Keil DJ. 2004. Diploid and polyploid cytotype distribution in *Melampodium cinereum* and *M. leucanthum* (Asteraceae, Heliantheae). *American Journal of Botany* **91**: 889–898.

Suda J, Krahulcová A, Trávníček P, Krahulec F. 2006. Ploidy level versus DNA ploidy level: an appeal for consistent terminology. *Taxon* **55**: 447–450.

Trávníček P, Kubátová B, Čurn V *et al.* 2011. Remarkable coexistence of multiple cytotypes of the *Gymnadenia conopsea* aggregate (the fragrant orchid): Evidence from flow cytometry. *Annals of Botany* **107**: 77–87.

Treier UA, Broennimann O, Normand S *et al.* 2009. Shift in cytotype frequency and niche space in the invasive plant *Centaurea maculosa*. *Ecology* **90**: 1366–1377.

Weiss-Schneeweiss H, Emadzade K, Jang TS, Schneeweiss GM. 2013. Evolutionary consequences, constraints and potential of polyploidy in plants. *Cytogenetic and Genome Research* **140**: 137–150.

Woo SL, Thomas AG, Peschken DP *et al.* 1991. The biology of Canadian weeds. 99. *Matricaria perforata* Mérat (Asteraceae). *Canadian Journal of Plant Science* **71**: 1101–1119.

Wood TE, Takebayashi N, Barker MS, Mayrose I, Greenspoon PB, Rieseberg LH. 2009. The frequency of polyploid speciation in vascular plants. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 13875–13879.

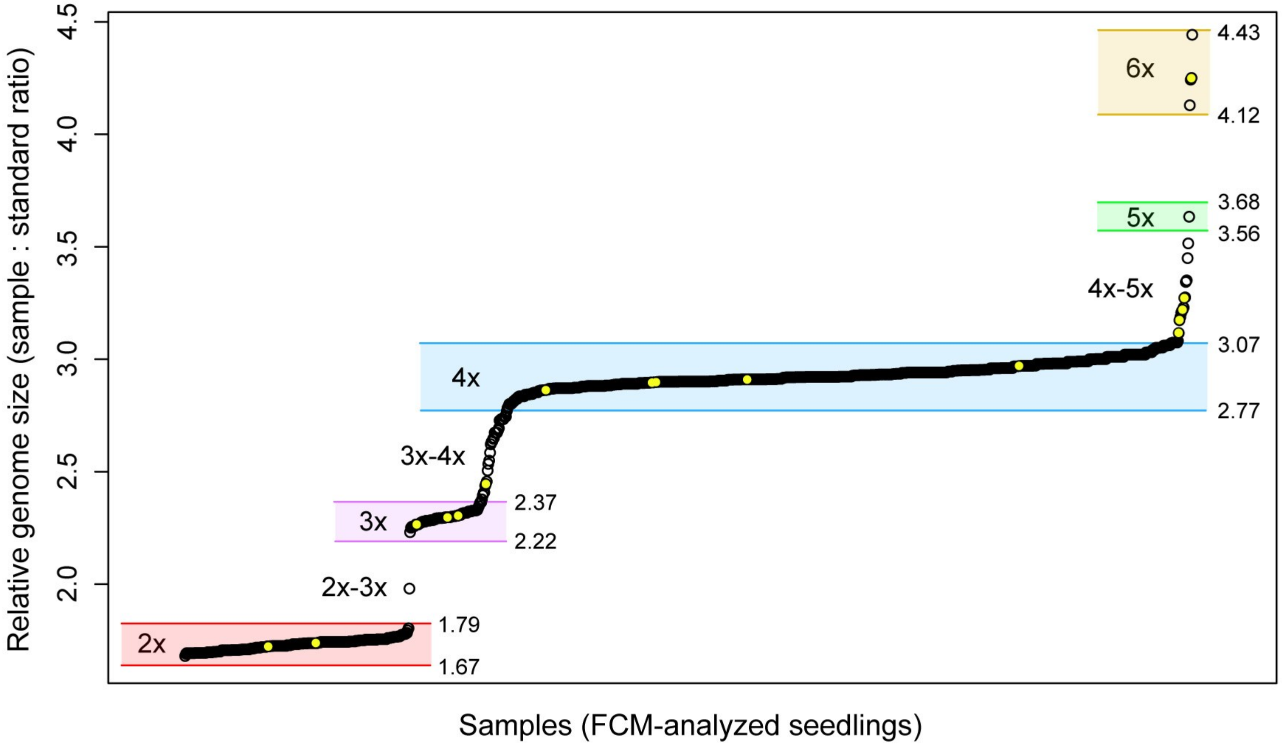

Figure S1. The more or less continuous distribution of relative genome size among 1,263 seedlings germinated from open-pollinated achenes collected in five mixed-ploidy and two uniform-ploidy populations. We used 16 plants with known chromosome numbers as reference points (yellow symbols) for setting arbitrary limits between five euploid cytotypes (2x, 3x, 4x, 5x, 6x) and three groups of aneuploids (2x–3x, 3x–4x, 4x–5x). Based on this division, the summary in Table 3 was prepared.

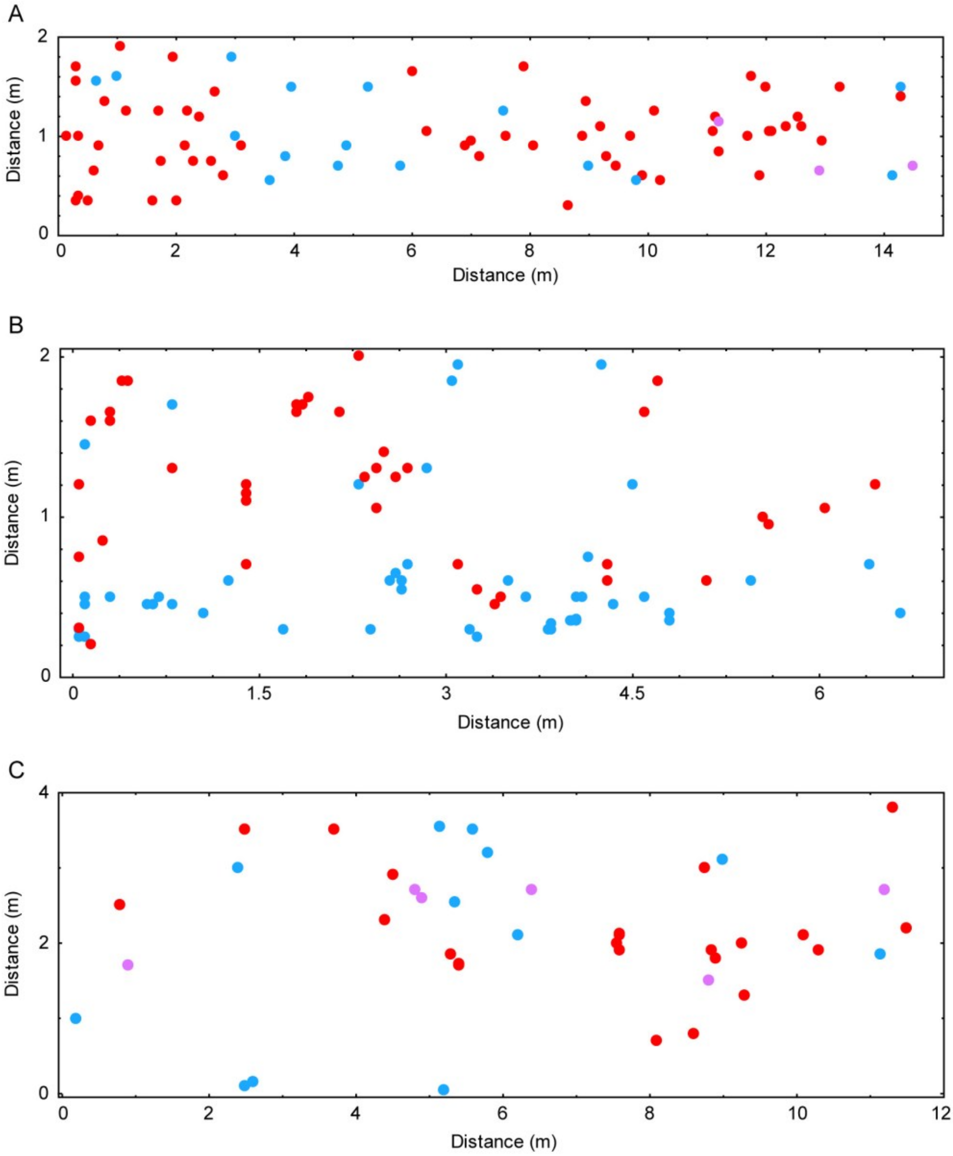

Figure S2. The occurrence of diploid (red), triploid (violet) and tetraploid (blue) individuals of *Tripleurospermum inodorum* within three study plots set in mixed-ploidy populations. (A) Population no. 150. (B) Population no. 889. (C) Population no. 916.

Table S1. List of 1,209 populations sampled during ploidy screening in *Tripleurospermum inodorum* at both Central-European and regional scales.

Population	Country[*]	Region[†]	The nearest town	Habitat type	Coordinates (WGS-84)	Date	Sample size[‡]	Ploidy composition			Other cytotypes[#]
								2x [%]	3x [%]	4x [%]	
1	CZ	MAR	Sítiny	grassland	50°01′53.5"N, 12°45′25.2"E	28.7.2011	8	100	0	0	–
2	CZ	–	Koudelka	roadside	50°04′57.0"N, 16°01′30.9"E	5.8.2011	1	0	0	100	–
3	CZ	–	Dolní Roveň	arable land	50°01′17.7"N, 15°57′49.9"E	4.8.2011	3	0	0	100	–
4	CZ	–	Holice	arable land	50°03′30.9"N, 15°59′13.5"E	4.8.2011	1	0	0	100	–
5	CZ	–	Praha-Pelc Tyrolka	roadside	50°06′52.8"N, 14°26′36.9"E	5.8.2011	1	0	0	100	–
6	CZ	–	Bavory	roadside	48°50′02.3"N, 16°37′38.2"E	16.8.2011	1	0	0	100	–
7	CZ	–	Brod nad Dyjí	roadside	48°52′34.2"N, 16°32′11.4"E	16.8.2011	2	0	0	100	–
8	CZ	–	Ivaň	roadside	48°55′10.0"N, 16°36′23.3"E	17.8.2011	2	0	0	100	–
9	CZ	–	Dobré Pole	roadside	48°49′31.8"N, 16°32′22.9"E	16.8.2011	1	0	0	100	–
10	CZ	–	Hlohovec	arable land	48°46′28.6"N, 16°46′34.7"E	18.8.2011	1	0	0	100	–
11	CZ	–	Hradec Králové	roadside	50°12′56.0"N, 15°49′49.0"E	14.8.2011	2	0	0	100	–
12	CZ	–	Jaroměř	railway	50°20′32.2"N, 15°54′40.6"E	12.8.2011	2	0	0	100	–
13	CZ	–	Nové Mlýny	roadside	48°51′42.4"N, 16°42′26.3"E	17.8.2011	1	0	0	100	–
14	CZ	–	Novosedly	roadside	48°50′11.5"N, 16°30′16.9"E	16.8.2011	2	0	0	100	–
15	CZ	–	Březí	roadside	48°49′27.8"N, 16°34′11.1"E	16.8.2011	2	0	0	100	–
16	CZ	–	Klentnice	roadside	48°51′20.8"N, 16°38′43.4"E	16.8.2011	1	0	0	100	–
17	CZ	–	Pec pod Sněžkou	roadside	50°42′27.2"N, 15°43′50.7"E	13.8.2011	2	100	0	0	–
18	CZ	–	Praha-Letňany	ruderal site	50°07′41.3"N, 14°30′56.9"E	26.8.2011	5	0	0	100	–
19	CZ	MAR	Prameny	roadside	50°02′31.0"N, 12°44′18.6"E	24.8.2011	1	100	0	0	–
20	CZ	MAR	Mnichov	roadside	50°02′21.3"N, 12°46′56.9"E	24.8.2011	1	0	0	100	–
21	CZ	MAR	Mnichov	roadside	50°02′11.2"N, 12°47′14.2"E	24.8.2011	8	0	0	100	–
22	CZ	MAR	Sítiny	grassland	50°01′58.2"N, 12°45′37.3"E	24.8.2011	25	46	2	52	–
23	CZ	MAR	Mariánské Lázně	ruderal site	49°57′31.8"N, 12°42′19.1"E	1.9.2011	1	0	0	100	–
24	CZ	–	Čestlice	roadside	50°00′15.3"N, 14°34′29.5"E	4.9.2011	1	0	0	100	–
25	CZ	–	Horní Stropnice	field drive	48°45′43.2"N, 14°45′01.5"E	13.11.2011	2	0	0	100	–
26	CZ	–	České Budějovice	roadside	48°58′42.6"N, 14°26′03.8"E	17.11.2011	5	0	0	100	–
27	CZ	–	Vlastiboř	arable land	49°15′11.2"N, 14°37′53.7"E	19.11.2011	8	0	0	100	–
28	CZ	–	Osek	ruderal site	50°37′11.2"N, 13°42′17.9"E	19.11.2011	6	0	0	100	–
29	CZ	–	Dlouhá Louka	roadside	50°38′41.4"N, 13°38′56.3"E	19.11.2011	2	0	0	100	–
30	CZ	–	Beroun	ruderal site	49°57′29.9"N, 14°04′31.7"E	24.4.2012	6	0	0	100	–
31	CZ	–	Valšov	ruderal site	49°55′41.7"N, 17°26′18.6"E	18.5.2012	10	0	0	100	–
32	CZ	–	Jihlava	ruderal site	49°25′00.7"N, 15°35′23.7"E	18.5.2012	10	0	0	100	–

33	CZ	–	Nová Pec	ruderal site	48°46´40.4"N, 13°52´03.0"E	18.5.2012	8	0	0	100	–
34	CZ	SOB	Chotěmice	arable land	49°15´43.9"N, 14°52´51.8"E	26.5.2012	7	86	0	14	–
35	CZ	–	Kamýk	arable land	50°33´24.2"N, 14°04´11.6"E	22.5.2012	5	0	0	100	–
36	CZ	–	České Budějovice	ruderal site	48°58´38.4"N, 14°26´46.6"E	7.6.2012	10	0	0	100	–
37	CZ	–	Hluboká u Borovan	pond bot	48°53´43.4"N, 14°39´55.2"E	7.6.2012	10	0	0	100	–
38	CZ	–	Dobronice	ruderal site	49°20´31.8"N, 14°29´41.5"E	7.6.2012	5	0	0	100	–
39	CZ	–	Hluboká n. Vltavou	pond bot	49°04´58.4"N, 14°24´35.5"E	7.6.2012	10	0	0	100	–
40	CZ	–	Tábor	pond bot	49°26´04.2"N, 14°40´05.4"E	19.6.2012	40	2.5	2.5	95	–
41	CZ	–	Mladá Vožice	roadside	49°31´53.8"N, 14°48´55.2"E	7.6.2012	5	0	0	100	–
42	CZ	–	Tábor	ruderal site	49°24´55.4"N, 14°40´20.0"E	19.6.2012	1	0	0	100	–
43	CZ	–	Tábor	field drive	49°25´25.3"N, 14°40´05.6"E	19.6.2012	12	0	0	100	–
44	CZ	–	Tábor	field drive	49°25´33.2"N, 14°40´01.0"E	19.6.2012	10	0	0	100	–
45	CZ	–	Kunžak-Lomy	arable land	49°06´36.4"N, 15°09´12.4"E	20.6.2012	2	0	0	100	–
46	CZ	MAR	Planá	ruderal site	49°51´41.5"N, 12°44´32.5"E	30.6.2012	10	50	10	40	–
47	CZ	–	Pastviny	grassland	50°16´43.5"N, 12°08´49.6"E	30.6.2012	10	0	0	100	–
48	CZ	–	Horní Slavkov	roadside	50°08´55.6"N, 12°48´38.3"E	30.6.2012	5	0	0	100	–
49	CZ	–	Svéradice	arable land	49°23´40.5"N, 13°44´38.5"E	30.6.2012	10	0	0	100	–
50	CZ	–	Čimelice	ruderal site	49°28´05.0"N, 14°04´34.5"E	30.6.2012	10	0	0	100	–
51	CZ	–	Pukňov	grassland	49°31´50.5"N, 14°07´54.3"E	30.6.2012	10	0	0	100	–
52	CZ	–	Uhelná	roadside	50°22´06.0"N, 16°59´29.6"E	11.7.2012	2	0	0	100	–
53	CZ	–	Horní Teplice	roadside	50°36´29.6"N, 16°09´58.9"E	13.7.2012	2	0	0	100	–
54	CZ	–	Horní Teplice	grassland	50°36´59.2"N, 16°10´38.4"E	13.7.2012	30	0	0	100	–
55	CZ	–	Horní Teplice	grassland	50°37´21.3"N, 16°10´28.0"E	13.7.2012	10	0	0	100	–
56	CZ	–	Loket	ruderal site	50°11´14.3"N, 12°45´03.1"E	10.7.2012	16	0	0	100	–
57	CZ	–	Loket	roadside	50°11´29.0"N, 12°45´48.2"E	10.7.2012	9	0	0	100	–
58	CZ	–	Hory	roadside	50°11´40.2"N, 12°48´33.7"E	10.7.2012	8	0	0	100	–
59	CZ	–	Doubí	grassland	50°12´19.2"N, 12°49´05.1"E	10.7.2012	34	0	0	100	–
60	CZ	–	Březová	roadside	50°12´11.9"N, 12°50´16.6"E	10.7.2012	8	0	0	100	–
61	CZ	–	Karlovy Vary	ruderal site	50°13´47.9"N, 12°51´44.4"E	10.7.2012	2	0	0	100	–
62	CZ	–	Sobotín	ruderal site	50°00´41.5"N, 17°05´29.7"E	19.7.2012	8	100	0	0	–
63	CZ	–	Malá Morávka	roadside	49°59´22.9"N, 17°19´03.3"E	7.8.2012	5	80	20	0	–
64	CZ	–	Bezděkov	roadside	49°46´36.0"N, 12°46´37.1"E	1.9.2012	5	0	0	100	–
65	CZ	–	Brod nad Tichou	fallow	49°49´11.8"N, 12°44´18.6"E	1.9.2012	9	0	0	100	–
66	CZ	MAR	Chodová Planá	roadside	49°54´15.9"N, 12°44´08.7"E	1.9.2012	6	17	17	66	–
67	CZ	MAR	Číhaná	roadside	50°00´55.7"N, 12°46´45.5"E	1.9.2012	5	0	0	100	–
68	CZ	MAR	Sítiny	roadside	50°01´15.8"N, 12°46´10.4"E	1.9.2012	4	25	0	75	–
69	CZ	MAR	Prameny	roadside	50°02´30.7"N, 12°43´30.0"E	1.9.2012	1	0	0	100	–
70	CZ	MAR	Prameny	roadside	50°02´54.7"N, 12°43´54.7"E	1.9.2012	1	0	0	100	–
71	CZ	MAR	Prameny	roadside	50°03´30.1"N, 12°43´56.5"E	1.9.2012	4	0	0	100	–

72	CZ	MAR	Prameny	grassland	50°03´41.4"N, 12°44´30.4"E	1.9.2012	13	23	15	62	–
73	CZ	MAR	Prameny	grassland	50°03´41.4"N, 12°44´15.4"E	1.9.2012	15	93	0	7	–
74	CZ	MAR	Nová Ves	roadside	50°04´18.1"N, 12°45´20.1"E	1.9.2012	8	0	0	100	–
75	CZ	MAR	Nová Ves	roadside	50°04´21.8"N, 12°47´05.7"E	1.9.2012	3	0	0	100	–
76	CZ	MAR	Planá	ruderal site	49°51´42.0"N, 12°43´49.7"E	30.7.2012	16	6	0	94	–
77	CZ	MAR	Planá	ruderal site	49°51´44.3"N, 12°44´26.2"E	30.7.2012	7	71	14.5	14.5	–
78	CZ	MAR	Planá	ruderal site	49°51´34.6"N, 12°44´28.6"E	30.7.2012	19	0	0	100	–
79	CZ	MAR	Planá	fallow	49°51´31.2"N, 12°44´34.7"E	30.7.2012	22	59	9	32	–
80	CZ	MAR	Brod nad Tichou	grassland	49°50´04.4"N, 12°44´45.0"E	30.7.2012	4	50	0	50	–
81	CZ	–	Brandýs nad Labem	arable land	50°10´32.1"N, 14°40´18.6"E	22.7.2012	7	0	0	100	–
82	CZ	–	Špindlerův mlýn	roadside	50°43´41.6"N, 15°36´02.2"E	21.7.2012	1	100	0	0	–
83	CZ	MAR	Mnichov	ruderal site	50°02´26.0"N, 12°46´40.1"E	9.9.2011	17	18	0	82	–
84	CZ	MAR	Sítiny	roadside	50°01´40.7"N, 12°45´37.1"E	1.9.2011	1	100	0	0	–
85	CZ	MAR	Sítiny	grassland	50°01´52.5"N, 12°45´32.0"E	1.9.2011	24	100	0	0	–
86	CZ	–	Raná	roadside	50°24´40.2"N, 13°46´43.7"E	6.9.2012	1	0	0	100	–
87	CZ	–	Hnojnice	arable land	50°25´59.2"N, 13°53´12.9"E	6.9.2012	6	0	0	100	–
88	CZ	–	Knobložka	arable land	50°32´21.7"N, 14°05´18.5"E	6.9.2012	1	0	0	100	–
89	CZ	–	Stráž nad Ohří	roadside	50°20´18.3"N, 13°03´13.8"E	8.9.2012	4	0	0	100	–
90	CZ	–	Vojkovice	roadside	50°17´48.0"N, 13°00´56.5"E	8.9.2012	5	0	0	100	–
91	CZ	–	Velichov	roadside	50°17´01.8"N, 13°00´15.0"E	8.9.2012	5	0	0	100	–
92	CZ	–	Ostrov	arable land	50°18´07.2"N, 12°58´49.0"E	8.9.2012	8	0	0	100	–
93	CZ	–	Hazlov	grassland	50°10´01.7"N, 12°16´00.7"E	8.9.2012	21	0	0	100	–
94	DE	–	Woja	fallow	50°15´26.2"N, 11°58´46.4"E	8.9.2012	23	100	0	0	–
95	DE	–	Bug	roadside	50°12´24.7"N, 11°51´47.6"E	9.9.2012	9	0	0	100	–
96	DE	–	Schüttersmühle	roadside	49°45´02.9"N, 11°25´39.3"E	9.9.2012	1	0	0	100	–
97	DE	–	Auerbach in der Ob.	roadside	49°41´24.3"N, 11°37´23.7"E	9.9.2012	4	25	0	75	–
98	DE	–	Edelsfeld	roadside	49°34´59.6"N, 11°40´58.8"E	9.9.2012	10	0	0	100	–
99	DE	–	Ensdorf	roadside	49°20´49.0"N, 11°55´43.4"E	9.9.2012	2	0	0	100	–
100	DE	–	Schmidmühlen	roadside	49°15´47.1"N, 11°55´50.9"E	9.9.2012	6	0	0	100	–
101	DE	–	Burglengenfeld	roadside	49°12´01.2"N, 12°01´39.7"E	9.9.2012	1	0	0	100	–
102	DE	–	Kreith	roadside	49°21´13.0"N, 12°04´38.2"E	9.9.2012	1	0	0	100	–
103	DE	–	Strassenhäuser	arable land	49°36´29.0"N, 12°19´46.1"E	9.9.2012	6	0	0	100	–
104	CZ	–	Kateřinské Chalupy	roadside	49°40´28.1"N, 12°36´09.6"E	9.9.2012	8	0	0	100	–
105	CZ	–	Mlýnec	roadside	49°41´38.4"N, 12°42´19.2"E	9.9.2012	8	0	0	100	–
106	CZ	–	Vysočany	arable land	49°41´58.5"N, 12°45´13.0"E	9.9.2012	10	40	0	60	–
107	CZ	–	Nová Hospoda	grassland	49°44´36.3"N, 12°46´51.2"E	9.9.2012	14	0	0	100	–
108	CZ	–	Rudná	roadside	50°00´39.3"N, 14°11´03.9"E	9.9.2012	4	0	0	100	–
109	CZ	SOB	Chotěmice	ruderal site	49°16´31.7"N, 14°52´33.4"E	18.8.2012	5	80	0	20	–
110	CZ	SOB	Chotěmice	arable land	49°15´45.6"N, 14°52´51.3"E	18.8.2012	5	100	0	0	–

111	CZ	–	Libchyně	roadside	50°21´24.1"N, 16°11´24.3"E	5.8.2012	3	100	0	0	–
112	CZ	–	Sendraž	roadside	50°22´01.5"N, 16°11´55.7"E	5.8.2012	1	0	0	100	–
113	CZ	–	Přerov	ruderal site	49°26´51.3"N, 17°26´38.3"E	3.8.2012	2	0	0	100	–
114	CZ	–	Olomouc	roadside	49°35´39.4"N, 17°16´34.9"E	3.8.2012	2	0	0	100	–
115	CZ	–	Olomouc	roadside	49°35´44.8"N, 17°16´28.2"E	3.8.2012	3	0	0	100	–
116	CZ	–	Běloves	ruderal site	50°25´24.6"N, 16°11´26.1"E	22.9.2012	6	0	0	100	–
117	CZ	–	Velemín	arable land	50°32´36.1"N, 13°58´25.8"E	13.11.2012	7	0	0	100	–
118	CZ	–	Březno	arable land	50°31´17.8"N, 13°58´36.1"E	13.11.2012	7	0	14	86	–
119	CZ	–	Podhorská ves	forest drive	48°39´37.3"N, 14°42´37.3"E	23.10.2012	5	0	0	100	–
120	CZ	–	Žofín	ruderal site	48°40´28.5"N, 14°41´39.1"E	23.10.2012	5	0	0	100	–
121	CZ	RAK	Kounov	arable land	50°13´22.2"N, 13°42´12.0"E	15.10.2012	6	100	0	0	–
122	CZ	–	Praha-Stodůlky	ruderal site	50°02´45.8"N, 14°18´12.7"E	20.11.2012	5	0	0	100	–
123	CZ	SOB	Planá nad Lužnicí	roadside	49°20´36.4"N, 14°43´09.6"E	20.11.2012	5	0	0	100	–
124	CZ	–	Praha-Bubny	railway	50°06´18.3"N, 14°26´18.8"E	3.10.2012	3	0	0	100	–
125	CZ	–	Český Těšín	railway	49°44´31.5"N, 18°37´19.1"E	3.10.2012	3	0	0	100	–
126	CZ	–	Krásný Les	roadside	50°46´03.9"N, 13°56´07.5"E	19.7.2012	5	0	0	100	–
127	CZ	–	Volary	roadside	48°53´31.0"N, 13°53´43.0"E	19.7.2012	10	0	0	100	–
128	CZ	–	Volary	grassland	48°53´10.0"N, 13°53´44.0"E	19.7.2012	8	0	0	100	–
129	CZ	–	Strunkovice n. Blan.	ruderal site	49°04´28.0"N, 14°03´00.5"E	19.7.2012	10	0	0	100	–
130	CZ	–	Bohušov	ruderal site	50°14´15.0"N, 17°43´00.0"E	12.7.2012	10	0	0	100	–
131	CZ	–	Ostrožská Nová Ves	roadside	49°00´08.0"N, 17°26´26.1"E	2.4.2013	1	0	0	100	–
132	CZ	–	Panenský Týnec	arable land	50°17´29.0"N, 13°55´00.4"E	22.5.2013	16	0	0	100	–
133	CZ	RAK	Žerotín	field drive	50°16´46.6"N, 13°54´48.0"E	22.5.2013	22	0	0	100	–
134	CZ	RAK	Milý	roadside	50°14´13.0"N, 13°52´04.5"E	22.5.2013	8	0	0	100	–
135	CZ	RAK	Bílichov	fallow	50°15´36.3"N, 13°55´03.7"E	22.5.2013	6	0	0	100	–
136	CZ	RAK	Zichovec-Samotín	arable land	50°16´10.4"N, 13°55´06.1"E	22.5.2013	4	0	0	100	–
137	CZ	–	Panenský Týnec	roadside	50°17´26.6"N, 13°55´42.1"E	22.5.2013	13	0	0	100	–
138	CZ	–	Napajedla	arable land	49°10´20.0"N, 17°29´32.1"E	14.5.2013	4	0	0	100	–
139	CZ	–	Krčmaň	arable land	49°31´29.2"N, 17°19´53.1"E	14.5.2013	9	0	0	100	–
140	CZ	–	Slatinky	ruderal site	49°32´42.0"N, 17°06´01.0"E	5.5.2013	10	0	0	100	–
141	CZ	–	Kobylí	arable land	48°55´46.0"N, 16°51´43.0"E	5.5.2013	10	0	0	100	–
142	CZ	–	Újezd u Brna	arable land	49°06´37.0"N, 16°46´00.0"E	5.5.2013	10	0	0	100	–
143	CZ	–	Kramolín	arable land	49°08´17.0"N, 16°07´20.0"E	5.5.2013	5	0	0	100	–
144	CZ	–	Cikháj	roadside	49°38´43.0"N, 15°58´03.0"E	3.11.2012	2	0	0	100	–
145	CZ	MAR	Velká Hleďsebe	roadside	49°57´13.9"N, 12°39´56.8"E	5.6.2013	8	0	0	100	–
146	CZ	MAR	Malá Hleďsebe	grassland	49°56´57.7"N, 12°39´18.5"E	5.6.2013	2	0	0	100	–
147	CZ	MAR	Malá Hleďsebe	grassland	49°56´57.7"N, 12°38´37.7"E	5.6.2013	1	0	0	100	–
148	CZ	MAR	Krásné	roadside	49°57´13.0"N, 12°36´55.4"E	5.6.2013	2	100	0	0	–
149	CZ	MAR	Tři Sekery	roadside	49°56´36.8"N, 12°37´00.2"E	5.6.2013	6	0	0	100	–

150	CZ	MAR	Tři Sekery	field drive	49°56´36.0"N, 12°37´45.7"E	5.6.2013	14	43	14	43	–
151	CZ	MAR	Panský Vrch	grassland	49°56´29.7"N, 12°38´48.1"E	5.6.2013	13	0	0	100	–
152	CZ	MAR	Drmoul	roadside	49°56´22.1"N, 12°40´04.3"E	5.6.2013	1	0	0	100	–
153	CZ	MAR	Drmoul	roadside	49°56´35.1"N, 12°40´20.6"E	5.6.2013	12	0	0	100	–
154	CZ	MAR	Mariánské Lázně	roadside	49°57´06.2"N, 12°42´56.9"E	6.6.2013	3	0	0	100	–
155	CZ	MAR	Stanoviště	roadside	49°56´40.4"N, 12°43´39.8"E	6.6.2013	2	0	0	100	–
156	CZ	MAR	Chotěnov	ruderal site	49°55´49.5"N, 12°43´57.7"E	6.6.2013	11	9	0	91	–
157	CZ	MAR	Holubín	roadside	49°55´16.9"N, 12°44´47.8"E	6.6.2013	3	0	0	100	–
158	CZ	MAR	Holubín	arable land	49°55´11.2"N, 12°44´57.7"E	6.6.2013	10	10	0	90	–
159	CZ	MAR	Dolní Kramolín	fallow	49°54´19.3"N, 12°45´15.6"E	6.6.2013	1	100	0	0	–
160	CZ	MAR	Chodová Planá	ruderal site	49°53´43.5"N, 12°44´46.1"E	6.6.2013	9	89	0	11	–
161	CZ	MAR	Chodová Planá	arable land	49°53´11.0"N, 12°43´38.5"E	6.6.2013	18	100	0	0	–
162	CZ	MAR	Chodová Planá	railway	49°52´59.4"N, 12°43´35.4"E	6.6.2013	8	0	0	100	–
163	CZ	–	Chudíř	pond bot	50°18´06.1"N, 15°00´14.4"E	7.6.2013	8	0	0	100	–
164	CZ	–	Nymburk	railway	50°11´35.4"N, 15°02´42.0"E	7.6.2013	6	0	0	100	–
165	CZ	–	Tachov	railway	49°48´07.9"N, 12°38´22.4"E	12.6.2013	1	0	0	100	–
166	CZ	–	Tachov	roadside	49°48´00.2"N, 12°38´06.7"E	12.6.2013	2	0	0	100	–
167	CZ	–	Světce	ruderal site	49°48´12.9"N, 12°36´29.9"E	12.6.2013	13	0	0	100	–
168	CZ	–	Světce	roadside	49°48´17.9"N, 12°36´23.6"E	12.6.2013	7	14	0	86	–
169	CZ	MAR	Svobodka	field drive	49°49´05.6"N, 12°36´18.1"E	12.6.2013	1	0	0	100	–
170	CZ	MAR	Ctiboř	ruderal site	49°49´14.3"N, 12°36´21.1"E	12.6.2013	29	0	0	100	–
171	CZ	MAR	Ctiboř	roadside	49°49´31.2"N, 12°36´23.9"E	12.6.2013	15	0	0	100	–
172	CZ	MAR	Ctiboř	grassland	49°49´47.7"N, 12°36´58.1"E	12.6.2013	19	0	0	100	–
173	CZ	MAR	Březí	arable land	49°50´02.0"N, 12°37´44.5"E	12.6.2013	5	0	0	100	–
174	CZ	MAR	Josefův Dvůr	forest drive	49°50´20.6"N, 12°39´38.4"E	12.6.2013	1	0	0	100	–
175	CZ	MAR	Josefův Dvůr	arable land	49°50´28.4"N, 12°40´34.8"E	12.6.2013	9	0	0	100	–
176	CZ	MAR	Nahý Újezdec	field drive	49°50´26.1"N, 12°40´54.0"E	12.6.2013	10	0	10	90	–
177	CZ	MAR	Nahý Újezdec	arable land	49°50´20.4"N, 12°41´30.4"E	12.6.2013	6	0	0	100	–
178	CZ	MAR	Planá	arable land	49°52´09.5"N, 12°45´31.9"E	13.6.2013	7	100	0	0	–
179	CZ	MAR	Planá	arable land	49°52´13.5"N, 12°45´58.2"E	13.6.2013	16	100	0	0	–
180	CZ	MAR	Kříženec	roadside	49°52´20.1"N, 12°46´23.2"E	13.6.2013	7	0	0	100	–
181	CZ	MAR	Kříženec	arable land	49°52´38.7"N, 12°46´44.5"E	13.6.2013	7	43	43	14	–
182	CZ	MAR	Kříženec	field drive	49°52´46.6"N, 12°47´16.3"E	13.6.2013	5	60	20	20	–
183	CZ	MAR	Catlov	forest drive	49°52´42.1"N, 12°48´20.8"E	13.6.2013	14	0	7	93	–
184	CZ	MAR	Catlov	roadside	49°52´16.1"N, 12°48´17.3"E	13.6.2013	1	0	0	100	–
185	CZ	MAR	Otín	forest drive	49°52´02.1"N, 12°48´20.5"E	13.6.2013	21	0	0	100	–
186	CZ	MAR	Otín	fallow	49°51´46.8"N, 12°48´05.7"E	13.6.2013	1	0	0	100	–
187	CZ	MAR	Otín	field drive	49°51´36.2"N, 12°47´50.4"E	13.6.2013	1	0	0	100	neo4x (3.58, CV = 1.55%)

188	CZ	MAR	Otín	arable land	49°51´30.5"N, 12°47´49.6"E	13.6.2013	2	0	0	100	–
189	CZ	MAR	Otín	roadside	49°51´29.4"N, 12°47´40.8"E	13.6.2013	2	0	50	50	4x-5x (3.14, CV = 1.65%)
190	CZ	MAR	Týnec	roadside	49°51´03.3"N, 12°46´16.2"E	13.6.2013	2	0	0	100	–
191	CZ	MAR	Týnec	arable land	49°51´15.3"N, 12°46´15.3"E	13.6.2013	19	0	0	100	–
192	CZ	MAR	Týnec	fallow	49°51´31.1"N, 12°46´09.0"E	13.6.2013	26	0	8	92	–
193	CZ	MAR	Planá	roadside	49°51´53.9"N, 12°44´04.3"E	13.6.2013	4	0	0	100	–
194	CZ	–	Stříbro	roadside	49°44´53.6"N, 12°59´57.8"E	16.6.2013	1	0	0	100	–
195	CZ	–	Stříbro	ruderal site	49°44´57.8"N, 12°59´57.8"E	16.6.2013	7	0	0	100	–
196	CZ	–	Stříbro	ruderal site	49°45´01.4"N, 12°59´50.9"E	16.6.2013	32	0	0	100	–
197	CZ	–	Stříbro	arable land	49°44´34.5"N, 12°59´17.3"E	16.6.2013	28	0	0	100	–
198	CZ	–	Stříbro	arable land	49°44´29.0"N, 12°59´02.6"E	16.6.2013	28	0	0	100	–
199	CZ	–	Vrbice u Stříbra	forest drive	49°44´30.9"N, 12°58´17.5"E	16.6.2013	8	0	0	100	–
200	CZ	–	Nový Mlýn	ruderal site	49°44´30.8"N, 12°57´19.4"E	16.6.2013	1	0	0	100	–
201	CZ	–	Milíkov	roadside	49°44´52.2"N, 12°56´56.4"E	16.6.2013	9	0	0	100	–
202	CZ	–	Milíkov	roadside	49°44´49.6"N, 12°56´20.2"E	16.6.2013	6	0	0	100	–
203	CZ	–	Jezerce	field drive	49°45´17.5"N, 12°56´23.8"E	16.6.2013	20	0	0	100	–
204	CZ	–	Jezerce	arable land	49°45´47.8"N, 12°56´20.8"E	16.6.2013	10	0	0	100	–
205	CZ	–	Otročín	field drive	49°46´14.4"N, 12°56´31.5"E	16.6.2013	11	0	0	100	–
206	CZ	–	Otročín	arable land	49°46´14.7"N, 12°56´00.0"E	16.6.2013	18	0	0	100	–
207	CZ	–	Svojšín	roadside	49°46´04.1"N, 12°54´32.3"E	16.6.2013	18	0	0	100	–
208	CZ	–	Svojšín	arable land	49°46´15.0"N, 12°54´08.2"E	16.6.2013	15	0	0	100	–
209	CZ	–	Svojšín	railway	49°45´48.3"N, 12°54´29.5"E	16.6.2013	25	0	0	100	–
210	CZ	–	Nynkov	arable land	49°45´37.7"N, 12°54´04.6"E	16.6.2013	18	0	0	100	–
211	CZ	–	Svojšín	roadside	49°45´45.7"N, 12°54´46.9"E	16.6.2013	4	0	0	100	–
212	CZ	–	Stříbro	railway	49°44´25.2"N, 12°59´58.0"E	16.6.2013	13	0	0	100	–
213	CZ	MAR	Poutnov	railway	50°01´07.8"N, 12°49´18.4"E	26.6.2013	13	0	0	100	–
214	CZ	MAR	Poutnov	roadside	50°00´52.5"N, 12°49´08.1"E	26.6.2013	5	0	0	100	–
215	CZ	MAR	Poutnov	roadside	50°00´39.2"N, 12°48´44.0"E	26.6.2013	37	30	3	67	–
216	CZ	MAR	Babice	field drive	50°00´27.5"N, 12°48´38.1"E	26.6.2013	11	36	0	64	–
217	CZ	MAR	Babice	roadside	50°00´20.7"N, 12°48´08.7"E	26.6.2013	8	75	0	25	–
218	CZ	MAR	Číhaná	roadside	50°00´42.1"N, 12°47´00.8"E	26.6.2013	4	0	50	50	–
219	CZ	MAR	Sítiny	roadside	50°01´02.4"N, 12°46´36.3"E	26.6.2013	2	100	0	0	–
220	CZ	MAR	Sítiny	ruderal site	50°01´37.8"N, 12°45´58.2"E	26.6.2013	9	78	11	11	–
221	CZ	MAR	Mnichov	grassland	50°02´02.0"N, 12°46´51.6"E	26.6.2013	1	100	0	0	–
222	CZ	MAR	Mnichov	arable land	50°02´04.8"N, 12°47´00.3"E	26.6.2013	17	6	0	94	–
223	CZ	MAR	Mariánské Lázně	roadside	49°57´23.5"N, 12°41´50.1"E	27.6.2013	8	0	0	100	–
224	CZ	MAR	Planá	arable land	49°52´26.7"N, 12°44´57.1"E	27.6.2013	6	100	0	0	–
225	CZ	MAR	Planá	arable land	49°52´42.0"N, 12°45´13.9"E	27.6.2013	18	100	0	0	–

226	CZ	MAR	Planá	roadside	49°52´59.6"N, 12°45´27.7"E	27.6.2013	4	50	25	25	–
227	CZ	MAR	Výškov	arable land	49°53´06.6"N, 12°45´35.5"E	27.6.2013	28	98	2	0	–
228	CZ	MAR	Výškov	arable land	49°53´22.5"N, 12°45´52.0"E	27.6.2013	19	89	11	0	–
229	CZ	MAR	Výškov	roadside	49°53´31.3"N, 12°46´09.7"E	27.6.2013	1	0	0	100	–
230	CZ	MAR	Výškov	grassland	49°53´43.8"N, 12°46´05.5"E	27.6.2013	5	80	0	20	–
231	CZ	MAR	Výškov	arable land	49°53´50.3"N, 12°46´06.5"E	27.6.2013	18	78	0	22	–
232	CZ	MAR	Mnichov	forest drive	50°02´17.1"N, 12°46´20.3"E	5.7.2013	1	0	0	100	–
233	CZ	MAR	Mnichov	grassland	50°01´53.4"N, 12°47´24.4"E	5.7.2013	2	50	50	0	–
234	CZ	MAR	Mnichov	roadside	50°01´45.4"N, 12°47´51.1"E	5.7.2013	13	0	0	100	–
235	CZ	MAR	Bohuslav	roadside	50°01´49.7"N, 12°48´12.3"E	5.7.2013	40	0	0	100	–
236	CZ	MAR	Bohuslav	roadside	50°02´05.5"N, 12°48´20.3"E	5.7.2013	8	38	0	62	–
237	CZ	MAR	Bohuslav	grassland	50°02´08.9"N, 12°48´16.4"E	5.7.2013	11	0	9	91	–
238	CZ	MAR	Bohuslav	grassland	50°02´04.0"N, 12°48´36.5"E	5.7.2013	20	0	0	100	–
239	CZ	MAR	Popovice	roadside	50°01´26.4"N, 12°48´21.5"E	5.7.2013	2	0	0	100	–
240	CZ	MAR	Poutnov	roadside	50°01´06.5"N, 12°49´05.4"E	5.7.2013	1	0	0	100	–
241	CZ	–	Plzeň-Litice	ruderal site	49°41´43.0"N, 13°20´49.0"E	2.6.2013	10	0	0	100	–
242	CZ	–	Arnoštov	roadside	48°53´38.0"N, 13°59´31.0"E	4.6.2013	5	0	0	100	–
243	CZ	–	Mičovice	arable land	48°58´33.0"N, 14°08´00.0"E	5.6.2013	10	0	0	100	–
244	CZ	–	Košatka	pasture	49°44´07.0"N, 18°09´07.0"E	12.6.2013	5	100	0	0	–
245	CZ	–	Karvinná-Darkov	ruderal site	49°50´08.0"N, 18°32´46.0"E	13.6.2013	10	0	0	100	–
246	CZ	–	Bohumín-Vrbice	ruderal site	49°53´07.0"N, 18°19´07.0"E	13.6.2013	5	0	0	100	–
247	CZ	–	Nový Bernštejn	arable land	50°33´04.5"N, 14°32´38.8"E	27.6.2013	6	0	0	100	–
248	CZ	–	Hubenov	field drive	49°23´44.4"N, 15°28´20.7"E	26.6.2013	5	0	0	100	–
249	CZ	–	Mirošov	field drive	49°23´06.6"N, 15°27´37.9"E	26.6.2013	6	0	0	100	–
250	CZ	–	Řídelov	roadside	49°14´17.6"N, 15°24´04.6"E	26.6.2013	2	0	0	100	–
251	CZ	–	Kostelec	roadside	49°21´20.6"N, 15°29´24.8"E	26.6.2013	3	0	0	100	–
252	CZ	–	Beroun-Jarov	ruderal site	49°56´24.8"N, 14°03´07.8"E	27.7.2013	10	0	0	100	–
253	CZ	–	Beroun	field drive	49°56´19.6"N, 14°04´13.8"E	27.7.2013	2	0	0	100	–
254	SK	–	Leles	pasture	48°28´38.5"N, 22°03´37.4"E	9.7.2013	5	0	0	100	–
255	SK	–	Spišské Podhradie	arable land	49°00´21.5"N, 20°43´27.0"E	8.7.2013	14	0	0	100	–
256	CZ	–	Sokolov	roadside	50°11´04.8"N, 12°38´26.4"E	5.9.2013	6	0	0	100	–
257	CZ	–	Sokolov	ruderal site	50°10´57.5"N, 12°38´31.1"E	5.9.2013	22	0	0	100	–
258	CZ	–	Sokolov	roadside	50°10´07.9"N, 12°40´31.1"E	5.9.2013	7	0	0	100	–
259	CZ	–	Sokolov	roadside	50°10´02.5"N, 12°40´39.8"E	5.9.2013	8	0	0	100	–
260	CZ	–	Hrušková	forest drive	50°08´54.3"N, 12°43´34.3"E	5.9.2013	17	0	0	100	–
261	CZ	MAR	Rovná	grassland	50°06´25.1"N, 12°43´36.8"E	5.9.2013	20	0	0	100	–
262	CZ	MAR	Rovná	ruderal site	50°06´07.4"N, 12°43´48.4"E	5.9.2013	12	0	0	100	–
263	CZ	MAR	Krásno	roadside	50°05´55.5"N, 12°43´46.5"E	5.9.2013	10	0	0	100	–
264	CZ	MAR	Krásno	roadside	50°05´23.2"N, 12°44´03.1"E	5.9.2013	7	0	0	100	–

265	CZ	MAR	Nová Ves u Sokolova	roadside	50°04´57.4"N, 12°44´56.3"E	5.9.2013	5	0	0	100	–
266	CZ	MAR	Nová Ves u Sokolova	ruderal site	50°04´52.0"N, 12°45´44.2"E	5.9.2013	31	0	0	100	–
267	CZ	MAR	Nová Ves u Sokolova	roadside	50°04´23.2"N, 12°45´26.7"E	5.9.2013	3	0	0	100	–
268	CZ	MAR	Nová Ves u Sokolova	grassland	50°04´15.8"N, 12°45´14.3"E	5.9.2013	14	0	7	93	–
269	CZ	MAR	Prameny	roadside	50°03´53.1"N, 12°44´47.3"E	5.9.2013	3	0	0	100	–
270	CZ	MAR	Prameny	roadside	50°03´01.4"N, 12°43´56.2"E	5.9.2013	4	0	0	100	–
271	CZ	MAR	Prameny	roadside	50°02´57.5"N, 12°44´22.3"E	5.9.2013	2	0	0	100	–
272	CZ	MAR	Mnichov	roadside	50°02´57.9"N, 12°45´17.6"E	5.9.2013	4	0	0	100	–
273	CZ	MAR	Závišín	roadside	49°58´34.1"N, 12°44´32.3"E	7.9.2013	2	0	0	100	–
274	CZ	MAR	Závišín	roadside	49°58´37.1"N, 12°45´49.9"E	7.9.2013	1	0	0	100	–
275	CZ	MAR	Závišín	forest drive	49°58´35.3"N, 12°45´55.9"E	7.9.2013	16	0	0	100	–
276	CZ	MAR	Závišín	forest drive	49°58´01.7"N, 12°46´02.3"E	7.9.2013	5	0	0	100	–
277	CZ	MAR	Ovesné Kladruby	roadside	49°57´30.6"N, 12°46´38.3"E	7.9.2013	1	0	0	100	–
278	CZ	MAR	Ovesné Kladruby	grassland	49°57´26.3"N, 12°46´55.1"E	7.9.2013	5	100	0	0	–
279	CZ	MAR	Ovesné Kladruby	railway	49°57´23.9"N, 12°47´05.3"E	7.9.2013	2	0	0	100	–
280	CZ	MAR	Ovesné Kladruby	roadside	49°57´17.6"N, 12°47´32.1"E	7.9.2013	3	0	0	100	–
281	CZ	MAR	Ovesné Kladruby	grassland	49°57´15.8"N, 12°48´01.0"E	7.9.2013	4	75	0	25	–
282	CZ	MAR	Mrázov	roadside	49°57´25.0"N, 12°48´31.1"E	7.9.2013	1	0	0	100	–
283	CZ	MAR	Mrázov	roadside	49°57´37.1"N, 12°49´22.9"E	7.9.2013	4	75	0	25	–
284	CZ	MAR	Mrázov	roadside	49°57´39.0"N, 12°49´52.4"E	7.9.2013	11	0	45	55	–
285	CZ	MAR	Mrázov	grassland	49°57´15.1"N, 12°50´14.4"E	7.9.2013	30	100	0	0	–
286	CZ	MAR	Klášter	roadside	49°57´31.7"N, 12°51´15.3"E	7.9.2013	19	42	0	58	–
287	CZ	MAR	Klášter Teplá	fallow	49°57´46.7"N, 12°52´08.6"E	7.9.2013	20	25	0	75	–
288	CZ	MAR	Klášter Teplá	roadside	49°58´04.9"N, 12°52´32.1"E	7.9.2013	8	37.5	25	37.5	–
289	CZ	MAR	Klášter Teplá	pasture	49°58´11.2"N, 12°52´03.3"E	7.9.2013	10	0	0	100	–
290	CZ	MAR	Teplá	roadside	49°58´19.5"N, 12°51´53.0"E	7.9.2013	5	0	0	100	–
291	CZ	MAR	Teplá	railway	49°58´29.0"N, 12°51´41.9"E	7.9.2013	5	0	0	100	–
292	CZ	SOB	Chotěmice	arable land	49°16´47.3"N, 14°51´37.8"E	31.8.2013	11	9	9	82	–
293	CZ	SOB	Chotěmice	arable land	49°16´26.4"N, 14°52´04.2"E	31.8.2013	9	89	0	11	–
294	CZ	SOB	Předboř	arable land	49°18´51.4"N, 14°51´21.7"E	31.8.2013	9	0	0	100	–
295	CZ	SOB	Chroustník	arable land	49°19´29.2"N, 14°50´29.0"E	31.8.2013	10	70	0	30	–
296	CZ	SOB	Brandlín	arable land	49°18´41.8"N, 14°47´19.8"E	31.8.2013	2	100	0	0	–
297	CZ	SOB	Deštná	grassland	49°15´25.5"N, 14°56´04.6"E	31.8.2013	5	80	0	20	–
298	CZ	SOB	Deštná	fallow	49°15´24.2"N, 14°56´14.2"E	31.8.2013	8	12.5	0	87.5	–
299	CZ	SOB	Deštná	grassland	49°15´24.0"N, 14°56´15.8"E	31.8.2013	9	0	0	100	–
300	CZ	SOB	Deštná	arable land	49°15´33.3"N, 14°56´16.4"E	31.8.2013	15	0	0	100	–
301	CZ	SOB	Deštná	roadside	49°15´36.6"N, 14°56´15.7"E	31.8.2013	1	0	0	100	–
302	CZ	SOB	Deštná	arable land	49°15´38.0"N, 14°56´11.0"E	31.8.2013	5	0	0	100	–
303	CZ	SOB	Drunče	arable land	49°17´36.4"N, 14°55´37.7"E	31.8.2013	13	0	0	100	–

304	CZ	SOB	Mnich	arable land	49°17´48.3"N, 14°57´29.8"E	1.9.2013	7	0	0	100	–
305	CZ	SOB	Mirotín	arable land	49°18´02.3"N, 14°58´58.0"E	1.9.2013	13	0	0	100	–
306	CZ	SOB	Bohdalín	arable land	49°18´13.3"N, 15°00´35.9"E	1.9.2013	36	0	0	100	–
307	CZ	SOB	Horní Radouň	roadside	49°15´49.3"N, 15°00´30.6"E	1.9.2013	15	0	0	100	–
308	CZ	SOB	Horní Radouň	grassland	49°15´25.1"N, 14°59´54.0"E	1.9.2013	5	0	80	20	–
309	CZ	SOB	Světce	arable land	49°15´59.3"N, 14°58´07.8"E	1.9.2013	9	0	11	89	–
310	CZ	SOB	Soběslav	roadside	49°15´28.7"N, 14°43´57.1"E	1.9.2013	17	0	0	100	–
311	CZ	–	Hojná Voda	roadside	48°43´44.0"N, 14°43´38.8"E	1.9.2013	2	0	0	100	–
312	CZ	–	Cheb	ruderal site	50°04´30.4"N, 12°22´43.2"E	18.9.2013	15	0	0	100	–
313	CZ	MAR	Dolní Žandov	railway	50°00´43.1"N, 12°32´23.8"E	18.9.2013	17	0	6	94	–
314	CZ	MAR	Brtná	roadside	50°00´38.2"N, 12°32´04.0"E	18.9.2013	2	0	0	100	–
315	CZ	MAR	Dolní Žandov	roadside	50°01´01.5"N, 12°32´59.7"E	18.9.2013	7	0	0	100	–
316	CZ	MAR	Dolní Žandov	roadside	50°00´46.6"N, 12°33´08.1"E	18.9.2013	1	0	0	100	–
317	CZ	MAR	Horní Žandov	fallow	50°00´18.0"N, 12°33´13.2"E	18.9.2013	5	0	0	100	–
318	CZ	MAR	Horní Žandov	roadside	50°00´03.7"N, 12°32´53.5"E	18.9.2013	2	0	0	100	–
319	CZ	MAR	Horní Žandov	pasture	49°59´55.9"N, 12°32´49.4"E	18.9.2013	14	0	0	100	–
320	CZ	MAR	Vysoká	roadside	49°59´43.4"N, 12°32´55.7"E	18.9.2013	3	0	0	100	–
321	CZ	MAR	Vysoká	ruderal site	49°58´56.2"N, 12°32´54.3"E	18.9.2013	2	0	0	100	–
322	CZ	MAR	Vysoká	pasture	49°58´54.5"N, 12°33´27.2"E	18.9.2013	4	50	25	25	–
323	CZ	MAR	Vysoká	pasture	49°58´53.8"N, 12°33´34.8"E	18.9.2013	26	12	15	73	–
324	CZ	MAR	Vysoká	arable land	49°59´04.7"N, 12°34´13.0"E	18.9.2013	23	0	0	100	–
325	CZ	MAR	Stará Voda	pasture	49°59´16.7"N, 12°34´53.5"E	18.9.2013	11	100	0	0	–
326	CZ	MAR	Stará Voda	pasture	49°59´23.8"N, 12°35´05.6"E	18.9.2013	15	100	0	0	–
327	CZ	MAR	Stará Voda	ruderal site	49°59´34.3"N, 12°35´22.1"E	18.9.2013	8	0	0	100	–
328	CZ	MAR	Stará Voda	roadside	49°59´30.0"N, 12°35´41.9"E	18.9.2013	5	0	0	100	–
329	CZ	MAR	Stará Voda	ruderal site	49°59´35.1"N, 12°36´17.4"E	18.9.2013	9	0	0	100	–
330	CZ	MAR	Lázně Kynžvart	railway	49°59´37.0"N, 12°36´47.3"E	18.9.2013	3	0	0	100	–
331	CZ	MAR	Lázně Kynžvart	roadside	50°00´00.8"N, 12°37´14.2"E	18.9.2013	1	0	0	100	–
332	CZ	MAR	Lázně Kynžvart	ruderal site	50°00´13.3"N, 12°37´16.4"E	18.9.2013	1	0	100	0	–
333	CZ	MAR	Stará Voda	roadside	49°59´39.7"N, 12°36´52.8"E	18.9.2013	3	0	0	100	–
334	CZ	MAR	Stará Voda	grassland	49°59´17.0"N, 12°35´44.3"E	8.10.2013	1	100	0	0	–
335	CZ	MAR	Stará Voda	roadside	49°59´08.6"N, 12°35´42.4"E	8.10.2013	9	11	0	89	–
336	CZ	MAR	Stará Voda	arable land	49°58´58.3"N, 12°35´40.2"E	8.10.2013	8	0	0	100	–
337	CZ	MAR	Stará Voda	arable land	49°58´54.0"N, 12°35´39.6"E	8.10.2013	21	52	0	48	–
338	CZ	MAR	Jedlová	pasture	49°58´45.5"N, 12°35´39.4"E	8.10.2013	7	0	0	100	–
339	CZ	MAR	Jedlová	roadside	49°58´44.8"N, 12°35´37.7"E	8.10.2013	4	0	0	100	–
340	CZ	MAR	Jedlová	roadside	49°58´31.2"N, 12°35´31.8"E	8.10.2013	4	0	0	100	–
341	CZ	MAR	Jedlová	pasture	49°58´32.3"N, 12°35´56.5"E	8.10.2013	24	0	0	100	–
342	CZ	MAR	Sekerské Chalupy	pasture	49°58´48.8"N, 12°36´38.1"E	8.10.2013	27	0	0	100	–

343	CZ	MAR	Sekerské Chalupy	roadside	49°58′50.6"N, 12°36′40.8"E	8.10.2013	5	0	0	100	–
344	CZ	MAR	Stará Voda	roadside	49°59′46.5"N, 12°36′14.4"E	8.10.2013	3	0	0	100	–
345	CZ	MAR	Lázně Kynžvart	ruderal site	50°00′29.4"N, 12°37′55.0"E	8.10.2013	1	0	0	100	–
346	CZ	–	Havlíčkův Brod	roadside	49°36′16.8"N, 15°34′51.4"E	25.4.2014	4	0	0	100	–
347	CZ	–	Žižkovo Pole	arable land	49°36′11.1"N, 15°44′17.0"E	25.4.2014	8	0	0	100	–
348	CZ	–	Polná	roadside	49°29′07.8"N, 15°43′19.7"E	25.4.2014	3	0	0	100	–
349	CZ	–	Přímělkov	roadside	49°20′08.1"N, 15°43′10.3"E	25.4.2014	1	0	0	100	–
350	CZ	–	Jaroměřice n. Rokyt.	ruderal site	49°07′53.8"N, 15°54′23.4"E	25.4.2014	6	0	17	83	–
351	CZ	–	Třebíč	roadside	49°12′59.7"N, 15°52′35.7"E	26.4.2014	2	0	0	100	–
352	CZ	–	Přelouč-Škudly	arable land	50°01′34.4"N, 15°32′05.5"E	13.4.2014	7	0	0	100	–
353	CZ	–	Přelouč	roadside	50°01′37.1"N, 15°32′30.7"E	13.4.2014	3	0	0	100	–
354	CZ	–	Všeborovice	arable land	50°14′45.8"N, 12°54′27.6"E	2.5.2014	14	0	0	100	–
355	CZ	–	Všeborovice	arable land	50°14′48.1"N, 12°54′50.4"E	2.5.2014	13	0	0	100	–
356	CZ	–	Bor	arable land	50°15′00.7"N, 12°54′58.8"E	2.5.2014	9	0	0	100	–
357	CZ	–	Bor	arable land	50°15′06.4"N, 12°55′03.0"E	2.5.2014	11	0	0	100	–
358	CZ	–	Bor	arable land	50°15′16.2"N, 12°55′07.5"E	2.5.2014	12	0	0	100	–
359	CZ	–	Bor	arable land	50°15′13.6"N, 12°55′18.9"E	2.5.2014	11	0	0	100	–
360	CZ	–	Bor	arable land	50°15′03.6"N, 12°55′26.5"E	2.5.2014	13	0	0	100	–
361	CZ	–	Bor	arable land	50°15′07.1"N, 12°55′48.9"E	2.5.2014	10	0	0	100	–
362	CZ	–	Bor	arable land	50°15′22.8"N, 12°55′48.1"E	2.5.2014	11	0	0	100	–
363	CZ	–	Bor	arable land	50°15′28.1"N, 12°56′06.7"E	2.5.2014	10	0	0	100	–
364	CZ	–	Žár	pond bot	48°48′06.7"N, 14°43′27.9"E	11.5.2014	11	0	0	100	–
365	CZ	RAK	Pnětluky	arable land	50°14′44.2"N, 13°41′13.7"E	11.5.2014	20	0	0	100	–
366	CZ	–	Starkoč	arable land	49°55′55.9"N, 15°29′59.5"E	8.7.2013	10	0	0	100	–
367	CZ	–	Běstvina-Javorka	arable land	49°50′14.2"N, 15°37′03.9"E	8.7.2013	10	100	0	0	–
368	CZ	–	Skuteč-Štěpánov	ruderal site	49°51′38.0"N, 15°59′47.0"E	4.9.2013	10	0	0	100	–
369	CZ	–	Častolovice	arable land	50°08′05.0"N, 16°09′41.0"E	4.9.2013	10	100	0	0	–
370	SK	–	Primovce	roadside	49°00′50.2"N, 20°22′51.8"E	4.10.2011	1	0	0	100	–
371	SK	–	Spišské Podhradie	grassland	48°59′51.4"N, 20°45′34.1"E	6.10.2011	3	0	0	100	–
372	DE	–	Bad Belzig	roadside	52°08′31.0"N, 12°35′23.0"E	6.6.2012	7	0	0	100	–
373	DE	–	Osterode am Harz	arable land	51°44′29.0"N, 10°12′53.0"E	6.6.2012	2	0	0	100	4x-5x (3.13, CV = 1.07%)
374	DE	–	Timmenrode	roadside	51°46′17.0"N, 11°00′17.0"E	6.6.2012	5	0	0	100	–
375	DE	–	Woffleben	ruderal site	51°33′24.0"N, 10°43′21.0"E	6.6.2012	8	0	0	100	–
376	SK	–	Jánovce	ruderal site	49°01′03.0"N, 20°25′49.0"E	7.8.2012	5	0	0	100	–
377	SK	–	Liptovský Ján	roadside	49°02′56.0"N, 19°40′35.0"E	7.8.2012	5	0	0	100	–
378	SK	–	Tatranská Javorina	forest drive	49°14′40.8"N, 20°09′39.9"E	7.8.2012	2	0	0	100	–
379	SK	–	Vysoké Tatry	forest drive	49°09′21.9"N, 20°09′26.9"E	7.8.2012	3	0	0	100	–
380	SK	–	Závod	arable land	48°32′09.7"N, 16°59′57.9"E	24.5.2014	6	0	0	100	–

381	DE	–	Luckau	arable land	51°51′35.6"N, 13°42′12.1"E	27.5.2014	2	0	0	100	4x-5x (3.10, CV = 1.33%)
382	CZ	–	Lanžhot	roadside	48°39′02.4"N, 16°55′43.2"E	19.5.2014	9	0	0	100	–
383	DE	–	Augsberg	roadside	49°25′26.5"N, 11°41′33.6"E	24.5.2014	1	0	0	100	–
384	DE	–	Herrieden	roadside	49°15′30.7"N, 10°29′58.0"E	24.5.2014	8	0	0	100	–
385	DE	–	Sattelweiler	roadside	49°10′55.8"N, 10°04′07.2"E	24.5.2014	2	0	0	100	–
386	CZ	–	Kateřinské Chalupy	roadside	49°40′42.9"N, 12°36′02.8"E	28.5.2014	1	0	0	100	–
387	CH	–	Kreuzlingen	roadside	47°39′18.3"N, 09°10′57.0"E	28.5.2014	12	0	0	100	–
388	DE	–	Konstanz	roadside	47°40′10.9"N, 09°10′31.0"E	29.5.2014	2	0	0	100	–
389	DE	–	Schwaig	roadside	48°21′17.7"N, 11°47′24.8"E	28.5.2014	2	0	0	100	–
390	DE	–	München	roadside	48°12′37.3"N, 11°37′10.4"E	1.6.2014	1	0	0	100	–
391	CZ	–	Lázně Bohdaneč	pond bot	50°05′29.8"N, 15°40′03.5"E	19.5.2014	22	0	0	100	–
392	CZ	–	Lázně Bohdaneč	arable land	50°06′01.9"N, 15°39′49.5"E	19.5.2014	11	0	0	100	–
393	CZ	–	Smiřice	roadside	50°17′59.2"N, 15°52′06.5"E	4.6.2014	2	0	0	100	–
394	AT	–	Obertauern	roadside	47°15′04.0"N, 13°32′40.0"E	27.8.2012	1	0	0	100	–
395	SK	–	Červená Skála	ruderal site	48°49′12.9"N, 20°08′05.9"E	17.6.2013	3	0	0	100	–
396	CZ	RAK	Nové Strašecí	arable land	50°09′04.3"N, 13°51′54.6"E	19.10.2013	12	75	8	17	–
397	CZ	RAK	Markvarec	roadside	50°16′37.6"N, 13°41′39.6"E	19.10.2013	15	0	0	100	–
398	CZ	RAK	Tuchořice	ruderal site	50°17′05.5"N, 13°39′49.9"E	19.10.2013	5	0	0	100	–
399	CZ	–	Cheb-Podhrad	roadside	50°03′27.3"N, 12°23′06.9"E	14.5.2014	5	0	0	100	–
400	CZ	–	Cheb	arable land	50°02′28.3"N, 12°23′21.3"E	14.5.2014	4	0	0	100	–
401	CZ	–	Cheb	arable land	50°02′52.3"N, 12°23′34.2"E	14.5.2014	14	0	0	100	–
402	CZ	–	Cheb	arable land	50°02′44.3"N, 12°23′32.5"E	14.5.2014	6	0	0	100	–
403	CZ	–	Malá Všeboř	arable land	50°02′34.7"N, 12°24′07.8"E	14.5.2014	1	0	0	100	–
404	CZ	–	Všeboř	arable land	50°02′30.1"N, 12°24′11.0"E	14.5.2014	1	0	0	100	–
405	CZ	–	Malá Všeboř	arable land	50°02′31.6"N, 12°24′19.8"E	14.5.2014	2	0	0	100	–
406	CZ	–	Malá Všeboř	roadside	50°02′38.4"N, 12°25′16.3"E	14.5.2014	2	0	0	100	–
407	CZ	–	Dolní Lipina	arable land	50°02′20.1"N, 12°25′36.6"E	14.5.2014	3	0	0	100	–
408	CZ	–	Dolní Lipina	roadside	50°02′15.5"N, 12°25′46.1"E	14.5.2014	6	0	0	100	–
409	CZ	–	Dolní Lipina	roadside	50°02′19.6"N, 12°26′06.2"E	14.5.2014	10	0	0	100	–
410	CZ	–	Lipová	roadside	50°02′12.9"N, 12°26′42.5"E	14.5.2014	8	0	0	100	–
411	CZ	–	Lipová	roadside	50°01′51.0"N, 12°27′15.5"E	14.5.2014	3	0	0	100	–
412	CZ	–	Dolní Lažany	arable land	50°01′50.2"N, 12°27′50.2"E	14.5.2014	6	0	0	100	–
413	CZ	–	Dolní Lažany	roadside	50°01′54.7"N, 12°28′22.7"E	14.5.2014	4	0	0	100	–
414	CZ	–	Horní Lažany	roadside	50°01′57.6"N, 12°29′06.1"E	14.5.2014	3	0	0	100	–
415	CZ	–	Horní Lažany	arable land	50°02′09.2"N, 12°29′05.9"E	14.5.2014	4	0	0	100	–
416	CZ	–	Horní Lažany	arable land	50°02′09.0"N, 12°29′06.3"E	14.5.2014	16	0	0	100	–
417	CZ	MAR	Salajna	ruderal site	50°01′37.0"N, 12°30′15.8"E	14.5.2014	3	0	0	100	–
418	CZ	MAR	Salajna	roadside	50°01′42.0"N, 12°30′35.5"E	14.5.2014	1	0	0	100	–

419	CZ	MAR	Salajna	arable land	50°01´41.7"N, 12°30´38.5"E	14.5.2014	14	0	0	100	–
420	CZ	MAR	Salajna	ruderal site	50°00´59.8"N, 12°31´21.2"E	14.5.2014	5	0	0	100	–
421	CZ	MAR	Dolní Žandov	ruderal site	50°00´43.1"N, 12°32´17.6"E	14.5.2014	6	0	0	100	–
422	CZ	MAR	Teplá	roadside	49°59´02.7"N, 12°51´56.4"E	23.5.2014	3	0	0	100	–
423	CZ	MAR	Teplá	roadside	49°59´26.6"N, 12°52´23.9"E	23.5.2014	2	0	0	100	–
424	CZ	MAR	Teplá	ruderal site	49°59´30.2"N, 12°52´28.3"E	23.5.2014	8	0	0	100	–
425	CZ	MAR	Teplá	field drive	49°59´29.7"N, 12°52´24.4"E	23.5.2014	5	40	0	60	–
426	CZ	MAR	Teplá	roadside	49°59´45.9"N, 12°52´31.2"E	23.5.2014	8	12.5	12.5	75	–
427	CZ	MAR	Teplá	ruderal site	49°59´50.3"N, 12°52´36.8"E	23.5.2014	8	0	0	100	–
428	CZ	MAR	Teplá	arable land	49°59´50.3"N, 12°52´36.8"E	23.5.2014	8	0	0	100	–
429	CZ	MAR	Teplá	field drive	50°00´12.1"N, 12°52´41.5"E	23.5.2014	7	0	0	100	–
430	CZ	MAR	Teplá	ruderal site	50°00´16.8"N, 12°52´39.6"E	23.5.2014	2	0	0	100	–
431	CZ	MAR	Otročín	field drive	50°01´04.8"N, 12°53´08.0"E	23.5.2014	10	70	20	10	–
432	CZ	MAR	Otročín	field drive	50°01´17.1"N, 12°53´26.4"E	23.5.2014	2	50	0	50	–
433	CZ	MAR	Otročín	grassland	50°01´51.2"N, 12°53´56.9"E	23.5.2014	12	50	0	50	–
434	CZ	MAR	Otročín	roadside	50°01´50.4"N, 12°54´14.9"E	23.5.2014	11	45	0	55	–
435	CZ	MAR	Otročín	roadside	50°01´51.6"N, 12°54´33.3"E	23.5.2014	6	0	50	50	–
436	CZ	MAR	Poseč	roadside	50°01´50.2"N, 12°54´56.9"E	23.5.2014	9	0	22	78	–
437	CZ	MAR	Poseč	grassland	50°02´02.5"N, 12°56´10.2"E	23.5.2014	9	0	0	100	–
438	CZ	–	Kosmová	pasture	50°02´00.0"N, 12°58´04.0"E	23.5.2014	7	0	0	100	–
439	CZ	–	Kosmová	ruderal site	50°02´06.5"N, 12°58´45.3"E	23.5.2014	11	0	0	100	–
440	CZ	–	Kosmová	roadside	50°02´05.2"N, 12°59´01.5"E	23.5.2014	14	0	0	100	–
441	CZ	–	Toužim	roadside	50°02´13.2"N, 12°59´40.8"E	23.5.2014	10	0	0	100	–
442	CZ	–	Toužim	roadside	50°02´45.9"N, 12°59´34.7"E	23.5.2014	13	0	0	100	–
443	CZ	–	Mostec	forest drive	50°04´38.7"N, 13°07´17.6"E	1.6.2014	16	0	0	100	–
444	CZ	–	Mostec	pasture	50°04´03.9"N, 13°06´48.1"E	1.6.2014	7	43	0	57	–
445	CZ	–	Štědrá	roadside	50°03´31.2"N, 13°06´44.6"E	1.6.2014	9	0	0	100	–
446	CZ	–	Lažany	arable land	50°03´36.9"N, 13°06´19.9"E	1.6.2014	12	0	0	100	–
447	CZ	–	Lažany	roadside	50°03´43.1"N, 13°06´06.3"E	1.6.2014	1	0	0	100	–
448	CZ	–	Lažany	roadside	50°03´50.4"N, 13°05´37.9"E	1.6.2014	3	66.5	0	33.5	–
449	CZ	–	Lažany	arable land	50°03´56.4"N, 13°04´55.5"E	1.6.2014	12	100	0	0	–
450	CZ	–	Brložec	roadside	50°03´59.7"N, 13°04´26.5"E	1.6.2014	9	0	11	89	–
451	CZ	–	Brložec	arable land	50°03´31.7"N, 13°04´17.3"E	1.6.2014	14	0	0	100	–
452	CZ	–	Brložec	roadside	50°03´35.0"N, 13°04´11.6"E	1.6.2014	5	0	40	60	–
453	CZ	–	Brložec	arable land	50°03´30.1"N, 13°03´54.2"E	1.6.2014	8	0	0	100	–
454	CZ	–	Smilov	arable land	50°03´28.0"N, 13°03´31.4"E	1.6.2014	16	0	0	100	–
455	CZ	–	Radyně	roadside	50°03´49.9"N, 13°02´14.9"E	1.6.2014	4	0	0	100	–
456	CZ	–	Radyně	field drive	50°03´46.5"N, 13°01´58.5"E	1.6.2014	9	0	0	100	–
457	CZ	–	Radyně	arable land	50°03´48.0"N, 13°01´40.6"E	1.6.2014	5	0	0	100	–

458	CZ	–	Radyně	arable land	50°03′50.3"N, 13°01′15.8"E	1.6.2014	6	0	0	100	–
459	CZ	–	Toužim	arable land	50°03′50.1"N, 13°00′27.1"E	1.6.2014	8	0	0	100	–
460	CZ	MAR	Tisová	grassland	50°02′19.0"N, 12°51′21.0"E	4.6.2014	1	0	0	100	–
461	CZ	MAR	Tisová	roadside	50°02′09.4"N, 12°51′45.4"E	4.6.2014	5	40	0	60	–
462	CZ	MAR	Tisová	field drive	50°02′06.1"N, 12°51′46.3"E	4.6.2014	14	0	0	100	–
463	CZ	MAR	Tisová	roadside	50°02′07.2"N, 12°52′19.1"E	4.6.2014	2	50	0	50	–
464	CZ	MAR	Otročín	field drive	50°02′06.9"N, 12°52′18.9"E	4.6.2014	3	33.3	0	66.7	–
465	CZ	MAR	Otročín	arable land	50°02′03.0"N, 12°53′00.7"E	4.6.2014	16	100	0	0	–
466	CZ	MAR	Otročín	roadside	50°01′55.4"N, 12°53′26.0"E	4.6.2014	7	0	0	100	–
467	CZ	MAR	Otročín	ruderal site	50°02′19.9"N, 12°53′16.4"E	4.6.2014	13	18.2	0	81.8	–
468	CZ	MAR	Brť	roadside	50°02′28.3"N, 12°53′28.3"E	4.6.2014	4	50	0	50	–
469	CZ	MAR	Brť	roadside	50°02′37.6"N, 12°53′27.7"E	4.6.2014	5	0	0	100	–
470	CZ	MAR	Brť	fallow	50°02′45.1"N, 12°53′42.9"E	4.6.2014	12	0	0	100	–
471	CZ	MAR	Brť	fallow	50°03′07.3"N, 12°54′26.8"E	4.6.2014	13	69	8	23	–
472	CZ	MAR	Brť	grassland	50°03′09.8"N, 12°54′38.5"E	4.6.2014	7	0	0	100	–
473	CZ	MAR	Sedlo	arable land	50°03′08.6"N, 12°55′18.2"E	4.6.2014	8	87.5	0	12.5	–
474	CZ	MAR	Sedlo	field drive	50°03′16.1"N, 12°56′02.2"E	4.6.2014	13	15	0	85	–
475	CZ	MAR	Sedlo	roadside	50°03′25.1"N, 12°56′34.2"E	4.6.2014	8	0	0	100	–
476	CZ	–	Útvina	roadside	50°04′09.1"N, 12°57′18.4"E	4.6.2014	4	0	0	100	–
477	CZ	–	Kryštofovo Údolí	ruderal site	50°45′46.0"N, 14°56′37.3"E	4.6.2014	13	0	0	100	–
478	CZ	–	Smečno	arable land	50°11′41.3"N, 14°02′04.0"E	24.5.2014	20	0	0	100	–
479	CZ	–	Jílové u Prahy	roadside	49°53′45.4"N, 14°29′11.5"E	18.5.2014	3	0	0	100	–
480	CZ	–	Moravský Písek	roadside	48°58′47.4"N, 17°19′02.1"E	8.6.2014	3	0	0	100	–
481	CZ	–	Velká Bukovina	grassland	50°44′12.3"N, 14°23′41.1"E	18.5.2014	15	0	0	100	–
482	CZ	–	Krásná Lípa	arable land	50°55′11.7"N, 14°30′11.4"E	18.5.2014	4	0	0	100	–
483	CZ	–	Lanžhot	arable land	48°37′40.7"N, 16°55′53.5"E	7.6.2014	19	0	0	100	–
484	CZ	–	Mutěnice	pond bot	48°54′20.9"N, 17°03′22.6"E	8.6.2014	10	0	0	100	–
485	CZ	–	Tvrdonice	field drive	48°45′13.9"N, 17°00′10.4"E	9.6.2014	20	0	0	100	–
486	CZ	–	Bohuslavice	ruderal site	50°18′49.1"N, 16°05′29.0"E	19.10.2014	11	0	0	100	–
487	CZ	–	Stádlec	roadside	49°22′02.7"N, 14°30′56.0"E	5.6.2014	16	0	6	94	–
488	CZ	–	Malšice	forest drive	49°21′36.4"N, 14°33′15.3"E	5.6.2014	1	0	0	100	–
489	CZ	–	Malšice	arable land	49°21′50.6"N, 14°34′08.9"E	5.6.2014	28	0	0	100	–
490	CZ	–	Dobronice	arable land	49°20′59.3"N, 14°29′58.5"E	3.6.2014	33	0	0	100	–
491	CZ	–	Dobronice	roadside	49°21′05.7"N, 14°30′42.7"E	4.6.2014	16	0	0	100	–
492	CZ	–	Dobronice	ruderal site	49°20′55.4"N, 14°30′49.0"E	5.6.2014	6	0	0	100	–
493	DE	–	Ilmenau	railway	50°41′00.2"N, 10°55′20.5"E	5.4.2014	3	0	0	100	–
494	DE	–	Koniesee	ruderal site	50°39′18.1"N, 11°08′11.6"E	5.4.2014	8	0	0	100	–
495	DE	–	Bebra-Blankenheim	roadside	50°56′21.1"N, 09°46′35.3"E	4.4.2014	4	0	0	100	–
496	CZ	–	Bzenec	ruderal site	48°58′23.7"N, 17°19′27.9"E	6.6.2014	10	0	0	100	–

497	CZ	–	Čížov	arable land	48°52´11.5"N, 15°51´42.3"E	12.6.2014	10	0	0	100	–
498	CZ	–	Drnholec	ruderal site	48°51´21.4"N, 16°29´13.4"E	9.6.2014	10	0	0	100	–
499	CZ	–	Dušejov	grassland	49°24´32.3"N, 15°26´01.2"E	5.6.2014	10	0	0	100	–
500	CZ	–	Havraníky	arable land	48°48´57.9"N, 16°00´34.8"E	10.6.2014	10	0	0	100	–
501	CZ	–	Ježov	arable land	49°37´06.1"N, 15°14´19.7"E	31.5.2014	10	0	0	100	–
502	CZ	–	Olbramkostel	pond bot	48°55´50.6"N, 15°57´34.6"E	13.6.2014	10	0	0	100	–
503	CZ	–	Plaveč	arable land	48°55´59.1"N, 16°04´27.6"E	11.6.2014	5	0	0	100	–
504	CZ	–	Sedlec	grassland	48°46´32.9"N, 16°41´54.4"E	9.6.2014	2	0	0	100	–
505	CZ	–	Senožaty	arable land	49°33´55.6"N, 15°11´32.8"E	31.5.2014	5	0	0	100	–
506	CZ	–	Vrbovec	arable land	48°46´45.4"N, 16°08´05.3"E	13.6.2014	5	0	0	100	–
507	CZ	–	Praha-Šeberov	pond bot	50°00´49.3"N, 14°30´50.1"E	16.6.2014	20	0	0	100	–
508	CZ	–	Bezděkov	ruderal site	49°24´48.9"N, 13°43´43.2"E	18.6.2014	7	0	0	100	–
509	CZ	–	Dnešice	pond bot	49°36´45.7"N, 13°16´21.3"E	18.6.2014	9	0	0	100	–
510	CZ	–	Toužim	arable land	50°03´45.9"N, 13°00´49.3"E	18.6.2014	15	34	0	66	5x (3.75, CV = 2.37%)
511	CZ	–	Diana	forest drive	49°37´07.4"N, 12°33´52.5"E	20.6.2014	8	0	0	100	–
512	CZ	–	Meclov	roadside	49°30´25.8"N, 12°52´41.7"E	20.6.2014	9	0	0	100	–
513	CZ	–	Kdyně	arable land	49°23´33.8"N, 13°01´27.5"E	20.6.2014	7	0	0	100	–
514	CZ	–	Mlázovy	arable land	49°18´39.2"N, 13°24´57.6"E	20.6.2014	13	0	0	100	–
515	CZ	–	Želnava	railway	48°48´25.9"N, 13°56´21.8"E	21.6.2014	1	0	0	100	–
516	CZ	–	Sušice	ruderal site	49°14´06.1"N, 13°31´04.7"E	22.6.2014	16	0	0	100	–
517	CZ	–	Ostrožská Nová Ves	arable land	49°00´03.1"N, 17°25´23.1"E	28.6.2014	7	0	0	100	–
518	CZ	–	Ostrožská Nová Ves	ruderal site	49°00´07.3"N, 17°25´07.9"E	28.6.2014	3	0	0	100	–
519	CZ	–	Smržovka	roadside	50°44´12.3"N, 15°14´37.7"E	18.6.2014	9	0	0	100	–
520	CZ	–	Smržovka	roadside	50°43´35.4"N, 15°14´28.1"E	18.6.2014	1	0	0	100	–
521	CZ	–	Nová Ves nad Nisou	roadside	50°43´15.6"N, 15°13´13.0"E	18.6.2014	4	0	0	100	–
522	CZ	–	Jablonecké Paseky	roadside	50°43´54.1"N, 15°11´26.0"E	18.6.2014	2	0	0	100	–
523	CZ	–	Liberec	roadside	50°45´47.9"N, 15°02´55.7"E	18.6.2014	12	0	0	100	–
524	CZ	–	Tvarožná	roadside	49°11´03.7"N, 16°45´34.0"E	20.6.2014	7	0	0	100	–
525	CZ	–	Valašská Polanka	roadside	49°14´58.5"N, 18°00´01.9"E	20.6.2014	5	0	0	100	–
526	SK	–	Púchov	roadside	49°07´42.1"N, 18°18´15.1"E	20.6.2014	2	0	0	100	–
527	SK	–	Východná	roadside	49°04´10.3"N, 19°54´58.9"E	20.6.2014	3	0	0	100	–
528	SK	–	Veľké Raškovce	fallow	48°33´45.3"N, 21°55´25.1"E	21.6.2014	10	0	0	100	–
529	HU	–	Streda n. Bodrogom	fallow	48°21´01.7"N, 21°44´17.2"E	22.6.2014	8	0	0	100	–
530	SK	–	Beša	fallow	48°31´39.6"N, 21°57´19.6"E	22.6.2014	7	0	0	100	–
531	SK	–	Vojany	arable land	48°34´17.6"N, 21°58´10.1"E	23.6.2014	13	0	0	100	–
532	SK	–	Levoča	roadside	49°02´56.9"N, 20°35´13.1"E	24.6.2014	1	0	0	100	–
533	SK	–	Hybe	roadside	49°03´28.4"N, 19°49´41.8"E	24.6.2014	8	0	0	100	–
534	SK	–	Galovany	ruderal site	49°05´34.1"N, 19°30´53.3"E	24.6.2014	16	0	0	100	–

535	SK	–	Rojkov	roadside	49°08′53.0"N, 19°09′37.7"E	24.6.2014	1	0	0	100	–
536	SK	–	Marček	ruderal site	49°15′56.4"N, 18°40′02.9"E	24.6.2014	3	0	0	100	–
537	CZ	–	Lidečko	railway	49°13′45.6"N, 18°02′40.4"E	24.6.2014	20	0	0	100	–
538	CZ	–	Zádveřice-Raková	roadside	49°12′44.5"N, 17°48′34.9"E	24.6.2014	1	0	0	100	–
539	CZ	–	Vyškov	arable land	49°15′37.9"N, 16°59′32.1"E	24.6.2014	13	0	0	100	–
540	CZ	–	Velké Meziříčí	roadside	49°21′14.3"N, 16°01′55.7"E	24.6.2014	6	0	0	100	–
541	CZ	–	Dunice	ruderal site	49°36′34.0"N, 15°09′14.1"E	24.6.2014	6	0	0	100	–
542	CZ	MAR	Planá	arable land	49°51′39.5"N, 12°44′15.5"E	3.7.2014	28	0	0	100	–
543	CZ	MAR	Brod nad Tichou	forest drive	49°50′56.8"N, 12°44′37.5"E	3.7.2014	2	50	0	50	–
544	CZ	MAR	Týnec	forest drive	49°50′50.8"N, 12°45′05.1"E	3.7.2014	11	36	0	64	–
545	CZ	MAR	Týnec	field drive	49°50′41.8"N, 12°45′21.6"E	3.7.2014	8	100	0	0	–
546	CZ	MAR	Týnec	roadside	49°50′46.0"N, 12°45′54.9"E	3.7.2014	3	0	0	100	–
547	CZ	MAR	Týnec	arable land	49°50′49.1"N, 12°46′11.7"E	3.7.2014	6	67	0	33	–
548	CZ	MAR	Planá	arable land	49°51′56.4"N, 12°45′42.4"E	3.7.2014	18	100	0	0	–
549	CZ	MAR	Křiženec	arable land	49°52′14.2"N, 12°45′57.9"E	3.7.2014	10	30	10	60	–
550	CZ	MAR	Křiženec	arable land	49°53′06.6"N, 12°46′19.8"E	5.7.2014	1	100	0	0	–
551	CZ	MAR	Křiženec	roadside	49°53′10.7"N, 12°46′17.8"E	5.7.2014	2	50	0	50	–
552	CZ	MAR	Výškov	fallow	49°53′19.8"N, 12°46′18.3"E	5.7.2014	21	100	0	0	–
553	CZ	MAR	Výškov	roadside	49°53′31.4"N, 12°45′58.0"E	5.7.2014	1	100	0	0	–
554	CZ	MAR	Výškov	roadside	49°53′33.2"N, 12°45′43.3"E	5.7.2014	10	90	0	10	–
555	CZ	MAR	Výškov	roadside	49°53′31.9"N, 12°45′12.8"E	5.7.2014	2	100	0	0	–
556	CZ	MAR	Chodová Planá	arable land	49°53′35.5"N, 12°44′39.4"E	5.7.2014	13	62	0	38	–
557	CZ	MAR	Chodová Planá	fallow	49°53′35.0"N, 12°44′22.1"E	5.7.2014	11	100	0	0	–
558	CZ	MAR	Chodová Planá	roadside	49°53′38.4"N, 12°44′06.0"E	5.7.2014	3	100	0	0	–
559	CZ	MAR	Planá	ruderal site	49°52′42.1"N, 12°43′40.3"E	5.7.2014	5	80	0	20	–
560	CZ	–	Větrný Jeníkov	arable land	49°28′26.1"N, 15°30′01.6"E	28.6.2014	10	0	0	100	–
561	CZ	–	Dobronín	arable land	49°29′12.3"N, 15°38′06.2"E	1.7.2014	10	0	0	100	–
562	CZ	–	Brzkov	arable land	49°31′56.4"N, 15°43′52.4"E	2.7.2014	10	0	0	100	–
563	CZ	–	Dobroutov	arable land	49°27′56.0"N, 15°45′21.5"E	2.7.2014	10	0	0	100	–
564	CZ	–	Jamné	arable land	49°26′03.8"N, 15°43′34.4"E	2.7.2014	5	0	0	100	–
565	CZ	RAK	Vroutek	ruderal site	50°10′32.8"N, 13°23′38.1"E	30.6.2014	21	0	0	100	–
566	CZ	RAK	Vroutek	fallow	50°10′12.8"N, 13°22′36.7"E	30.6.2014	21	0	0	100	–
567	CZ	RAK	Žihle	arable land	50°02′34.1"N, 13°23′14.8"E	1.7.2014	16	0	0	100	–
568	CZ	RAK	Blatno	arable land	50°05′25.0"N, 13°24′16.0"E	1.7.2014	21	62	5	33	–
569	CZ	–	Bražec	grassland	50°10′09.7"N, 13°02′07.4"E	2.7.2014	17	47	0	53	–
570	CZ	–	Bražec	pasture	50°10′33.5"N, 13°03′13.0"E	2.7.2014	9	0	0	100	–
571	CZ	–	Mašťov	forest drive	50°15′50.1"N, 13°16′17.4"E	3.7.2014	13	0	0	100	–
572	CZ	–	Dobřenec	arable land	50°15′11.4"N, 13°15′28.1"E	3.7.2014	21	0	0	100	–
573	CZ	RAK	Podbořany	grassland	50°13′02.4"N, 13°23′20.9"E	4.7.2014	24	0	0	100	–

574	CZ	–	Obědné	arable land	49°52´40.7"N, 17°02´48.0"E	6.7.2014	5	100	0	0	–
575	CZ	–	Jedlová	roadside	50°50´34.3"N, 14°33´33.8"E	25.6.2014	7	0	0	100	–
576	CZ	–	Naděje	forest drive	50°48´43.0"N, 14°38´18.4"E	25.6.2014	6	0	0	100	–
577	CZ	–	Harrachov	roadside	50°46´14.3"N, 15°28´22.7"E	26.6.2014	15	0	0	100	–
578	CZ	RAK	Podbořany	arable land	50°13´41.4"N, 13°23´00.5"E	1.7.2014	9	0	0	100	–
579	CZ	RAK	Zlovědice	arable land	50°15´49.0"N, 13°23´28.3"E	3.7.2014	10	0	0	100	–
580	CZ	–	Lhotka u Berouna	ruderal site	49°59´47.4"N, 14°05´58.7"E	3.7.2014	10	0	0	100	–
581	CZ	–	Liberec	roadside	50°46´45.4"N, 15°04´33.5"E	4.7.2014	3	0	0	100	–
582	CZ	–	Rokycany	roadside	49°44´19.3"N, 13°35´44.3"E	4.7.2014	2	0	0	100	–
583	CZ	RAK	Široké Třebčice	ruderal site	50°16´54.6"N, 13°23´10.8"E	3.7.2014	6	0	0	100	–
584	CZ	RAK	Vroutek	roadside	50°10´39.3"N, 13°23´05.1"E	30.6.2014	27	0	0	100	–
585	CZ	–	Žilina	arable land	50°07´00.6"N, 14°00´29.9"E	28.6.2014	15	0	0	100	–
586	CZ	RAK	Sýkořice	fallow	50°02´22.5"N, 13°56´46.4"E	28.6.2014	14	0	0	100	–
587	CZ	RAK	Všetaty	arable land	50°02´29.3"N, 13°44´10.2"E	28.6.2014	6	83	0	17	–
588	CZ	RAK	Panoší Újezd	arable land	50°01´42.8"N, 13°42´15.0"E	28.6.2014	8	0	0	100	–
589	CZ	RAK	Václavy	arable land	50°04´13.1"N, 13°35´26.3"E	28.6.2014	14	86	7	7	–
590	CZ	RAK	Přílepy	arable land	50°07´56.9"N, 13°38´09.9"E	28.6.2014	16	19	13	68	–
591	CZ	RAK	Deštnice	fallow	50°13´15.5"N, 13°35´18.7"E	28.6.2014	16	87	0	13	–
592	CZ	RAK	Tuchořice	arable land	50°17´21.3"N, 13°39´10.4"E	28.6.2014	10	0	0	100	–
593	CZ	RAK	Domoušice	field drive	50°13´28.2"N, 13°43´56.9"E	28.6.2014	15	20	0	80	–
594	CZ	RAK	Hředle	field drive	50°11´27.4"N, 13°44´28.1"E	28.6.2014	16	0	0	100	–
595	CZ	RAK	Mšecké Žehrovice	fallow	50°10´26.2"N, 13°54´42.0"E	28.6.2014	15	7	0	93	–
596	CZ	–	Lenora	roadside	48°55´22.4"N, 13°48´12.8"E	21.6.2014	5	0	0	100	–
597	CZ	–	Černíč	arable land	49°08´01.6"N, 15°27´12.1"E	17.6.2014	8	100	0	0	–
598	CZ	–	Zlatá Koruna	grassland	48°51´06.9"N, 14°21´12.0"E	1.7.2014	5	0	0	100	–
599	CZ	–	Červený Hrádek	arable land	49°07´03.0"N, 15°32´25.0"E	7.7.2014	11	0	0	100	–
600	CZ	–	Hostkovice	roadside	49°04´56.2"N, 15°22´58.3"E	20.10.2012	2	0	0	100	–
601	CZ	–	Hospříz	arable land	49°08´05.1"N, 15°03´47.1"E	20.10.2012	11	0	0	100	–
602	CZ	SOB	Lodhéřov	ruderal site	49°12´32.5"N, 14°56´38.3"E	20.10.2012	10	80	0	20	–
603	CZ	SOB	Červená Lhota	arable land	49°15´16.6"N, 14°53´45.8"E	20.10.2012	7	43	0	57	–
604	CZ	SOB	Deštná	arable land	49°15´44.5"N, 14°55´58.7"E	20.10.2012	22	91	0	9	–
605	CZ	–	Hořice	arable land	50°21´31.3"N, 15°37´09.9"E	22.6.2014	7	0	0	100	–
606	CZ	–	Nepomyšl	arable land	50°13´08.7"N, 13°19´36.6"E	2.6.2014	12	0	0	100	–
607	CZ	–	Sedlec u Radonic	arable land	50°15´59.0"N, 13°13´48.0"E	2.6.2014	12	0	0	100	–
608	CZ	–	Radošov	grassland	50°10´26.5"N, 13°08´13.3"E	1.6.2014	15	0	0	100	–
609	CZ	–	Hradiště	grassland	50°12´57.0"N, 13°06´50.0"E	3.7.2014	5	0	0	100	–
610	CZ	–	Valeč	roadside	50°10´45.1"N, 13°15´46.3"E	1.6.2014	3	0	0	100	–
611	CZ	–	Albeřice	pasture	50°10´16.4"N, 13°09´47.1"E	1.6.2014	8	0	0	100	–
612	CZ	RAK	Blatno	field drive	50°05´55.7"N, 13°22´42.4"E	2.6.2014	7	71	0	29	–

613	CZ	–	Tis u Blatna	forest drive	50°04´37.0"N, 13°21´02.0"E	2.6.2014	3	0	0	100	–
614	CZ	RAK	Vidhostice	arable land	50°09´15.6"N, 13°22´20.5"E	2.6.2014	9	0	0	100	–
615	CZ	–	Lubenec	ruderal site	50°07´56.0"N, 13°18´57.0"E	2.6.2014	6	0	0	100	–
616	CZ	RAK	Široké Třebčice	fallow	50°16´54.9"N, 13°23´13.2"E	3.6.2014	5	0	0	100	–
617	CZ	RAK	Mory	field drive	50°16´34.6"N, 13°24´40.5"E	3.6.2014	6	0	0	100	–
618	CZ	RAK	Vysoké Třebušice	arable land	50°15´17.7"N, 13°23´59.1"E	3.6.2014	9	0	0	100	–
619	CZ	–	Chyše	arable land	50°06´08.8"N, 13°13´22.3"E	3.6.2014	12	0	0	100	–
620	CZ	–	Hradiště	arable land	50°12´09.8"N, 13°11´12.1"E	4.6.2014	10	0	0	100	–
621	CZ	–	Podbořanský Rohoz.	forest drive	50°12´23.9"N, 13°15´01.3"E	4.6.2014	10	0	0	100	–
622	CZ	RAK	Velká Černoc	arable land	50°12´03.1"N, 13°34´57.2"E	3.6.2014	9	0	0	100	–
623	CZ	RAK	Vlkov	arable land	50°11´18.8"N, 13°32´55.0"E	3.6.2014	9	0	0	100	–
624	CZ	RAK	Soběchleby	arable land	50°13´24.0"N, 13°31´47.2"E	3.7.2014	10	0	0	100	–
625	CZ	–	Radechov	fallow	50°16´48.8"N, 13°16´29.8"E	4.7.2014	5	0	0	100	–
626	CZ	–	Mnichov	field drive	49°18´25.3"N, 13°50´36.6"E	10.7.2014	10	0	0	100	–
627	CZ	–	Domanice	arable land	49°18´01.6"N, 13°54´56.5"E	10.7.2014	10	0	0	100	–
628	CZ	–	Leskovice	pond bot	49°19´17.1"N, 13°54´55.1"E	10.7.2014	10	0	20	80	–
629	CZ	–	Vestec	arable land	49°50´28.1"N, 15°34´57.5"E	8.7.2014	6	33.3	0	66.7	–
630	CZ	–	Pařížov	arable land	49°49´51.7"N, 15°34´28.1"E	8.7.2014	10	0	0	100	–
631	CZ	–	Drhotín	arable land	49°49´09.2"N, 15°36´58.1"E	9.7.2014	10	50	0	50	–
632	CZ	–	Hoješín	field drive	49°48´37.3"N, 15°38´51.2"E	9.7.2014	9	100	0	0	–
633	CZ	–	Hoješín	arable land	49°49´00.1"N, 15°38´41.4"E	9.7.2014	4	50	0	50	–
634	CZ	–	Javorka	arable land	49°50´07.0"N, 15°37´18.8"E	9.7.2014	21	29	0	71	–
635	CZ	–	Seč	roadside	49°50´02.7"N, 15°38´10.5"E	9.7.2014	21	95	0	5	–
636	CZ	–	Bojanov	arable land	49°50´57.3"N, 15°41´50.9"E	9.7.2014	38	35	0	65	–
637	CZ	–	Polánka	arable land	49°49´41.0"N, 15°44´18.6"E	9.7.2014	7	0	0	100	–
638	CZ	–	Chloumek	arable land	49°46´47.0"N, 15°44´18.2"E	9.7.2014	7	72	14	14	–
639	CZ	–	Libice n. Doubravou	arable land	49°44´23.1"N, 15°42´59.5"E	9.7.2014	15	100	0	0	–
640	CZ	–	Chotěboř	roadside	49°42´33.7"N, 15°39´29.4"E	9.7.2014	17	76	0	24	–
641	CZ	–	Dolní Krupá	roadside	49°38´59.9"N, 15°36´03.6"E	9.7.2014	18	78	0	22	–
642	CZ	–	Šmolovy	arable land	49°35´30.6"N, 15°32´51.4"E	9.7.2014	32	3	0	97	–
643	CZ	–	Rozkoš	arable land	49°32´50.4"N, 15°23´58.5"E	9.7.2014	20	95	0	5	–
644	CZ	–	Dehtáře	fallow	49°28´49.1"N, 15°16´40.0"E	9.7.2014	23	0	0	100	–
645	CZ	–	Pelhřimov	fallow	49°24´58.6"N, 15°13´56.4"E	9.7.2014	25	0	0	100	–
646	CZ	–	Sázava	fallow	49°23´47.4"N, 15°19´31.1"E	9.7.2014	19	0	0	100	–
647	CZ	–	Pavlov	roadside	49°23´45.0"N, 15°15´06.8"E	9.7.2014	25	0	0	100	–
648	CZ	SOB	Kamenice nad Lipou	arable land	49°18´32.4"N, 15°04´40.9"E	9.7.2014	13	23	0	77	–
649	CZ	SOB	Gabrielka	arable land	49°17´03.0"N, 15°03´28.6"E	9.7.2014	15	20	0	80	–
650	CZ	SOB	Bohdalín	roadside	49°18´48.2"N, 15°00´00.5"E	9.7.2014	12	0	0	100	–
651	CZ	SOB	Mirotín	grassland	49°18´14.2"N, 14°58´30.3"E	9.7.2014	29	0	0	100	–

652	CZ	SOB	Mnich	arable land	49°17′32.7"N, 14°57′33.9"E	9.7.2014	22	0	0	100	–
653	CZ	–	Cítov	pasture	50°22′18.8"N, 14°23′27.3"E	19.7.2014	10	0	0	100	–
654	CZ	–	Nižbor	ruderal site	50°00′02.1"N, 14°00′21.0"E	17.7.2014	3	0	0	100	–
655	CZ	MAR	Hamrníky	ruderal site	49°56′56.2"N, 12°41′43.5"E	14.7.2014	21	0	0	100	–
656	CZ	MAR	Drmoul	arable land	49°56′22.3"N, 12°39′27.9"E	14.7.2014	6	100	0	0	–
657	CZ	MAR	Horní Kramolín	roadside	49°58′51.9"N, 12°47′13.9"E	15.7.2014	2	100	0	0	–
658	CZ	MAR	Horní Kramolín	roadside	49°58′44.8"N, 12°47′43.5"E	15.7.2014	10	0	0	100	–
659	CZ	MAR	Služetín	roadside	49°59′07.8"N, 12°48′04.8"E	15.7.2014	4	25	50	25	–
660	CZ	MAR	Služetín	roadside	49°59′30.3"N, 12°48′00.9"E	15.7.2014	6	50	0	50	–
661	CZ	MAR	Služetín	roadside	49°59′32.0"N, 12°48′18.4"E	15.7.2014	8	0	0	100	–
662	CZ	MAR	Hoštěc	roadside	49°59′33.5"N, 12°48′51.1"E	15.7.2014	6	66	17	17	–
663	CZ	MAR	Hoštěc	roadside	49°59′33.4"N, 12°50′03.4"E	15.7.2014	9	0	0	100	–
664	CZ	MAR	Hoštěc	arable land	49°59′33.6"N, 12°50′29.4"E	15.7.2014	6	100	0	0	–
665	CZ	MAR	Hoštěc	arable land	49°59′34.1"N, 12°50′32.8"E	15.7.2014	19	95	0	5	–
666	CZ	MAR	Rankovice	roadside	49°59′41.4"N, 12°51′06.0"E	15.7.2014	6	66.7	0	33.3	–
667	CZ	MAR	Teplá	roadside	49°59′07.8"N, 12°51′35.3"E	15.7.2014	4	100	0	0	–
668	CZ	MAR	Teplá	roadside	49°57′53.2"N, 12°51′44.1"E	15.7.2014	2	50	0	50	–
669	CZ	MAR	Teplá	arable land	49°57′54.1"N, 12°51′26.2"E	15.7.2014	16	0	0	100	–
670	CZ	MAR	Teplá	arable land	49°57′53.8"N, 12°51′27.4"E	15.7.2014	39	0	0	100	–
671	CZ	MAR	Mrázov	arable land	49°58′01.2"N, 12°50′51.5"E	15.7.2014	14	86	7	7	–
672	CZ	MAR	Mrázov	arable land	49°58′11.0"N, 12°50′11.7"E	15.7.2014	8	50	50	0	–
673	CZ	MAR	Velká Hleďsebe	ruderal site	49°57′51.9"N, 12°40′21.9"E	16.7.2014	7	86	14	0	–
674	CZ	MAR	Valy u Mar. Lázní	roadside	49°58′31.7"N, 12°39′31.0"E	16.7.2014	2	100	0	0	–
675	CZ	MAR	Valy u Mar. Lázní	roadside	49°58′57.9"N, 12°38′50.9"E	16.7.2014	2	100	0	0	–
676	CZ	MAR	Valy u Mar. Lázní	roadside	49°59′20.0"N, 12°38′35.0"E	16.7.2014	10	0	0	100	–
677	CZ	MAR	Lázně Kynžvart	roadside	49°59′39.4"N, 12°38′44.0"E	16.7.2014	2	100	0	0	–
678	CZ	MAR	Lázně Kynžvart	roadside	50°00′19.7"N, 12°37′50.7"E	16.7.2014	1	0	0	100	–
679	CZ	MAR	Lázně Kynžvart	ruderal site	49°59′57.1"N, 12°37′37.2"E	16.7.2014	16	0	0	100	–
680	CZ	MAR	Lázně Kynžvart	ruderal site	49°59′40.1"N, 12°37′18.5"E	16.7.2014	2	0	0	100	–
681	CZ	MAR	Stará voda	fallow	49°59′10.3"N, 12°34′35.0"E	16.7.2014	13	15	15	70	–
682	CZ	MAR	Vysoká	roadside	49°59′11.9"N, 12°33′26.2"E	16.7.2014	4	25	0	75	–
683	CZ	MAR	Stará voda	fallow	49°59′37.9"N, 12°34′10.8"E	16.7.2014	18	0	0	100	–
684	CZ	MAR	Stará voda	roadside	49°59′40.7"N, 12°34′35.8"E	16.7.2014	7	0	0	100	–
685	CZ	MAR	Krásný lán	roadside	49°57′59.3"N, 12°37′03.5"E	16.7.2014	4	50	0	50	–
686	CZ	MAR	Krásné	fallow	49°57′12.7"N, 12°36′47.4"E	16.7.2014	14	93	7	0	–
687	CZ	MAR	Bečov nad Teplou	roadside	50°05′00.7"N, 12°49′38.4"E	17.7.2014	1	100	0	0	–
688	CZ	MAR	Bečov nad Teplou	forest drive	50°04′49.7"N, 12°49′02.3"E	17.7.2014	2	0	0	100	–
689	CZ	MAR	Starý dvůr	roadside	50°05′06.5"N, 12°48′50.3"E	17.7.2014	3	100	0	0	–
690	CZ	MAR	Starý dvůr	pasture	50°05′18.4"N, 12°48′19.4"E	17.7.2014	21	0	0	100	–

691	CZ	MAR	Nová Ves	pasture	50°05´05.7"N, 12°47´01.9"E	17.7.2014	11	0	0	100	–
692	CZ	MAR	Nová Ves	roadside	50°05´01.9"N, 12°46´07.6"E	17.7.2014	4	0	0	100	–
693	CZ	MAR	Prameny	roadside	50°02´39.8"N, 12°43´15.2"E	17.7.2014	3	0	0	100	–
694	CZ	MAR	Prameny	roadside	50°02´21.7"N, 12°42´35.9"E	17.7.2014	5	40	0	60	–
695	CZ	MAR	Kladská	roadside	50°01´40.2"N, 12°40´27.7"E	17.7.2014	1	0	0	100	–
696	CZ	MAR	Mariánské lázně	forest drive	50°00´06.4"N, 12°41´01.2"E	17.7.2014	1	0	0	100	–
697	CZ	MAR	Drmoul	roadside	49°56´17.0"N, 12°39´22.3"E	18.7.2014	16	0	0	100	–
698	CZ	MAR	Panský vrh	roadside	49°56´18.8"N, 12°38´48.3"E	18.7.2014	12	100	0	0	–
699	CZ	MAR	Panský vrh	roadside	49°56´04.7"N, 12°38´19.8"E	18.7.2014	7	86	0	14	–
700	CZ	MAR	Tři sekery u Tachova	arable land	49°56´04.6"N, 12°37´36.4"E	18.7.2014	12	100	0	0	–
701	CZ	MAR	Plánská Huť	roadside	49°55´51.3"N, 12°36´59.3"E	18.7.2014	3	100	0	0	–
702	CZ	MAR	Plánská Huť	arable land	49°55´42.1"N, 12°36´48.8"E	18.7.2014	9	100	0	0	–
703	CZ	MAR	Chodovská Huť	ruderal site	49°55´13.2"N, 12°36´31.0"E	18.7.2014	7	0	0	100	–
704	CZ	MAR	Chodovská Huť	roadside	49°54´46.4"N, 12°35´56.8"E	18.7.2014	1	100	0	0	–
705	CZ	MAR	Broumov	forest drive	49°54´18.4"N, 12°35´48.8"E	18.7.2014	4	100	0	0	–
706	CZ	MAR	Broumov	ruderal site	49°53´39.7"N, 12°36´12.8"E	18.7.2014	16	0	0	100	–
707	CZ	MAR	Broumov	roadside	49°53´09.0"N, 12°37´36.0"E	18.7.2014	1	0	100	0	–
708	CZ	MAR	Broumov	arable land	49°53´12.0"N, 12°37´47.5"E	18.7.2014	6	100	0	0	–
709	CZ	MAR	Zadní Chodov	roadside	49°53´26.5"N, 12°39´19.7"E	18.7.2014	2	50	0	50	–
710	CZ	MAR	Zadní Chodov	fallow	49°53´10.8"N, 12°39´32.8"E	18.7.2014	14	21	7	72	–
711	CZ	MAR	Zadní Chodov	arable land	49°52´55.2"N, 12°39´29.5"E	18.7.2014	10	100	0	0	–
712	CZ	MAR	Chodský Újezd	roadside	49°51´57.3"N, 12°39´14.5"E	18.7.2014	7	100	0	0	–
713	CZ	MAR	Chodský Újezd	roadside	49°51´42.8"N, 12°39´08.9"E	18.7.2014	1	100	0	0	–
714	CZ	MAR	Horní Jadruž	grassland	49°51´52.6"N, 12°39´40.4"E	18.7.2014	5	80	0	20	–
715	CZ	MAR	Horní Jadruž	arable land	49°51´58.5"N, 12°39´47.9"E	18.7.2014	11	91	0	9	–
716	CZ	MAR	Horní Jadruž	ruderal site	49°52´00.8"N, 12°40´11.0"E	18.7.2014	2	0	0	100	–
717	CZ	MAR	Horní Jadruž	roadside	49°52´04.9"N, 12°40´35.3"E	18.7.2014	13	0	0	100	–
718	CZ	MAR	Dolní Jadruž	roadside	49°51´50.9"N, 12°41´40.2"E	18.7.2014	10	0	50	50	–
719	CZ	MAR	Dolní Jadruž	pond bot	49°51´49.2"N, 12°41´49.9"E	18.7.2014	17	0	0	100	–
720	CZ	MAR	Chodský Újezd	arable land	49°51´38.6"N, 12°42´03.2"E	18.7.2014	1	0	0	100	–
721	CZ	MAR	Chodský Újezd	roadside	49°51´29.5"N, 12°42´11.2"E	18.7.2014	1	0	0	100	–
722	CZ	MAR	Planá	ruderal site	49°52´17.5"N, 12°44´01.9"E	18.7.2014	8	0	0	100	–
723	CZ	MAR	Svatá Anna	fallow	49°52´30.0"N, 12°43´32.9"E	18.7.2014	13	0	0	100	–
724	CZ	MAR	Svatá Anna	roadside	49°52´34.4"N, 12°43´15.7"E	18.7.2014	10	0	0	100	–
725	CZ	MAR	Svatá Anna	arable land	49°52´37.5"N, 12°42´34.2"E	18.7.2014	13	77	0	23	–
726	CZ	MAR	Kyjov	pond bot	49°52´51.7"N, 12°41´45.2"E	18.7.2014	3	33.3	0	66.7	–
727	CZ	MAR	Kyjov	arable land	49°52´59.2"N, 12°41´32.3"E	18.7.2014	5	100	0	0	–
728	CZ	MAR	Kyjov	roadside	49°53´05.7"N, 12°41´06.6"E	18.7.2014	7	0	0	100	–
729	CZ	MAR	Kyjov	roadside	49°53´11.2"N, 12°40´51.4"E	18.7.2014	4	75	0	25	–

730	CZ	MAR	Kyjov	arable land	49°53′12.7"N, 12°40′35.3"E	18.7.2014	7	0	0	100	–
731	CZ	MAR	Zadní Chodov	ruderal site	49°53′55.7"N, 12°39′43.8"E	18.7.2014	10	100	0	0	–
732	CZ	MAR	Zadní Chodov	pond bot	49°53′56.7"N, 12°39′48.2"E	18.7.2014	12	42	0	58	–
733	CZ	MAR	Zadní Chodov	arable land	49°54′03.9"N, 12°40′07.5"E	18.7.2014	13	0	0	100	–
734	CZ	MAR	Horní Ves	arable land	49°54′27.8"N, 12°40′05.2"E	18.7.2014	12	58	0	42	–
735	CZ	MAR	Trstěnice	ruderal site	49°55′11.5"N, 12°40′34.9"E	18.7.2014	5	0	0	100	–
736	CZ	MAR	Trstěnice	arable land	49°55′16.8"N, 12°40′36.4"E	18.7.2014	12	50	8	42	–
737	CZ	–	Borovná	roadside	49°09′52.1"N, 15°23′41.3"E	21.7.2014	20	0	0	100	–
738	CZ	–	Budčeves	arable land	50°18′37.9"N, 15°15′16.6"E	17.7.2014	9	0	0	100	–
739	CZ	–	Vrbice	arable land	50°05′28.3"N, 16°14′54.9"E	18.7.2014	10	100	0	0	–
740	CZ	–	Kluč	roadside	50°03′34.6"N, 16°13′50.5"E	19.7.2014	11	0	0	100	–
741	CZ	–	Merklovice	arable land	50°06′26.5"N, 16°18′04.3"E	19.7.2014	12	0	0	100	–
742	CZ	–	Praha-Kunratice	pond bot.	50°00′33.1"N, 14°29′49.0"E	20.7.2014	10	0	0	100	–
743	CZ	–	Holubovská Bašta	pond bot.	49°00′13.6"N, 14°18′01.6"E	8.7.2014	3	0	0	100	–
744	CZ	–	Strýčice	arable land	49°00′28.4"N, 14°16′36.2"E	9.7.2014	6	0	0	100	–
745	CZ	–	Hluboká n. Vltavou	railway	49°02′02.7"N, 14°25′07.3"E	10.8.2014	2	0	0	100	–
746	CZ	–	Klášter	pond bot.	49°01′26.6"N, 15°09′52.3"E	10.8.2014	5	0	0	100	–
747	CZ	–	Olešná	arable land	49°26′48.5"N, 15°16′40.9"E	13.7.2014	10	0	0	100	–
748	CZ	SOB	Kamenice nad Lipou	arable land	49°18′35.9"N, 15°04′40.9"E	13.7.2014	10	0	0	100	–
749	CZ	–	Jindřichův Hradec	roadside	49°08′52.3"N, 15°01′18.3"E	13.7.2014	10	0	0	100	–
750	CZ	–	Stráž nad Nežárkou	arable land	49°04′03.2"N, 14°53′20.5"E	13.7.2014	10	0	0	100	–
751	CZ	–	Štěpánovice	roadside	48°59′45.7"N, 14°41′02.8"E	13.7.2014	10	0	0	100	–
752	CZ	MAR	Vlkovice	ruderal site	49°57′03.6"N, 12°44′01.4"E	1.8.2014	3	0	0	100	–
753	CZ	MAR	Vlkovice	pasture	49°56′40.2"N, 12°44′19.1"E	1.8.2014	8	87.5	0	12.5	–
754	CZ	MAR	Stanoviště	field drive	49°56′40.0"N, 12°44′11.2"E	1.8.2014	3	0	0	100	–
755	CZ	MAR	Martinov	fallow	49°56′00.3"N, 12°45′06.9"E	1.8.2014	6	0	0	100	–
756	CZ	MAR	Pístov	forest drive	49°55′48.0"N, 12°45′23.1"E	1.8.2014	1	0	0	100	–
757	CZ	MAR	Pístov	pasture	49°55′27.3"N, 12°45′51.4"E	1.8.2014	3	33.3	0	66.7	–
758	CZ	MAR	Dolní Kramolín	roadside	49°54′53.9"N, 12°45′13.7"E	1.8.2014	10	50	0	50	–
759	CZ	MAR	Dolní Kramolín	roadside	49°54′31.8"N, 12°44′11.9"E	1.8.2014	16	0	0	100	–
760	CZ	MAR	Chodová Planá	arable land	49°53′56.5"N, 12°44′02.8"E	1.8.2014	8	0	0	100	–
761	CZ	MAR	Planá	roadside	49°51′43.4"N, 12°43′03.0"E	1.8.2014	5	60	0	20	–
762	CZ	MAR	Chodský Újezd	roadside	49°52′15.6"N, 12°39′23.6"E	1.8.2014	10	0	0	100	–
763	CZ	MAR	Sítiny	forest drive	50°01′00.6"N, 12°42′58.2"E	2.8.2014	1	0	0	100	–
764	CZ	MAR	Prameny	roadside	50°03′12.3"N, 12°43′47.9"E	2.8.2014	21	0	0	100	–
765	CZ	MAR	Prameny	grassland	50°03′45.4"N, 12°44′28.1"E	2.8.2014	10	100	0	0	–
766	CZ	MAR	Babice	field drive	50°00′15.4"N, 12°48′35.4"E	2.8.2014	6	0	0	100	–
767	CZ	MAR	Babice	roadside	50°00′10.0"N, 12°48′24.6"E	2.8.2014	20	10	0	90	–
768	CZ	MAR	Číhaná	roadside	50°00′01.2"N, 12°48′05.1"E	2.8.2014	2	0	0	100	–

769	CZ	MAR	Služetín	pasture	49°59´50.9"N, 12°47´48.5"E	2.8.2014	13	54	0	46	–
770	CZ	MAR	Služetín	forest drive	49°59´48.8"N, 12°47´50.1"E	2.8.2014	15	75	0	25	–
771	CZ	MAR	Na Říčce	arable land	49°57´14.0"N, 12°52´49.0"E	3.8.2014	9	11	0	89	–
772	CZ	MAR	Na Říčce	roadside	49°57´05.9"N, 12°52´51.6"E	3.8.2014	23	0	0	100	–
773	CZ	MAR	Křepkovice	roadside	49°56´51.4"N, 12°52´51.0"E	3.8.2014	3	0	0	100	–
774	CZ	MAR	Křepkovice	roadside	49°56´34.8"N, 12°52´41.6"E	3.8.2014	1	0	0	100	–
775	CZ	MAR	Beranovka	field drive	49°56´24.8"N, 12°50´57.4"E	3.8.2014	17	12	18	70	–
776	CZ	MAR	Beranovka	pasture	49°56´24.2"N, 12°50´56.2"E	3.8.2014	10	100	0	0	–
777	CZ	MAR	Křepkovice	arable land	49°56´30.8"N, 12°52´48.3"E	3.8.2014	3	0	33.3	66.7	–
778	CZ	MAR	Křepkovice	field drive	49°56´26.3"N, 12°52´45.6"E	3.8.2014	5	20	60	20	–
779	CZ	MAR	Hanov	forest drive	49°55´32.0"N, 12°52´36.2"E	3.8.2014	6	33	0	67	–
780	CZ	MAR	Hanov	roadside	49°55´05.9"N, 12°52´30.5"E	3.8.2014	5	20	0	80	2x-3x (1.90, CV = 1.48%)
781	CZ	MAR	Hanov	roadside	49°54´50.8"N, 12°52´16.0"E	3.8.2014	11	36	36	28	–
782	CZ	MAR	Hanov	ruderal site	49°54´37.1"N, 12°52´08.7"E	3.8.2014	5	0	20	80	–
783	CZ	MAR	Hanov	roadside	49°54´35.6"N, 12°52´03.8"E	3.8.2014	5	0	0	100	–
784	CZ	MAR	Hanov	roadside	49°54´27.6"N, 12°51´39.8"E	3.8.2014	5	0	0	100	–
785	CZ	MAR	Hostíčkov	ruderal site	49°54´22.7"N, 12°51´09.0"E	3.8.2014	8	0	0	100	–
786	CZ	MAR	Hostíčkov	forest drive	49°54´17.4"N, 12°50´12.6"E	3.8.2014	3	0	0	100	–
787	CZ	MAR	Hostíčkov	roadside	49°54´13.9"N, 12°49´43.3"E	3.8.2014	5	0	0	100	–
788	CZ	MAR	Hostíčkov	roadside	49°54´02.0"N, 12°49´14.1"E	3.8.2014	1	100	0	0	–
789	CZ	MAR	Hostíčkov	field drive	49°54´36.1"N, 12°49´41.9"E	3.8.2014	1	0	0	100	–
790	CZ	MAR	Boněnov	field drive	49°54´46.8"N, 12°49´28.8"E	3.8.2014	2	0	0	100	–
791	CZ	MAR	Boněnov	grassland	49°54´54.6"N, 12°48´22.5"E	3.8.2014	1	100	0	0	–
792	CZ	MAR	Boněnov	roadside	49°55´04.6"N, 12°48´26.6"E	3.8.2014	2	0	0	100	–
793	CZ	MAR	Výškovice	roadside	49°55´41.3"N, 12°48´41.7"E	3.8.2014	2	0	0	100	–
794	CZ	MAR	Výškovice	grassland	49°55´49.6"N, 12°48´46.6"E	3.8.2014	6	83.3	16.7	0	–
795	CZ	MAR	Výškovice	pasture	49°55´50.5"N, 12°48´38.4"E	3.8.2014	6	100	0	0	–
796	CZ	MAR	Pístov	forest drive	49°55´24.7"N, 12°46´46.2"E	3.8.2014	1	0	0	100	–
797	CZ	MAR	Pístov	roadside	49°54´54.9"N, 12°45´53.4"E	3.8.2014	3	0	0	100	–
798	CZ	MAR	Dolní Kramolín	roadside	49°54´58.8"N, 12°43´46.5"E	3.8.2014	2	0	0	100	–
799	CZ	MAR	Skláře	roadside	49°55´30.7"N, 12°42´56.0"E	3.8.2014	2	0	50	50	–
800	CZ	MAR	Mariánské lázně	roadside	49°56´32.8"N, 12°42´30.1"E	3.8.2014	8	0	0	100	–
801	CZ	MAR	Sítiny	forest drive	50°00´44.2"N, 12°45´02.0"E	19.8.2014	4	0	0	100	–
802	CZ	MAR	Sítiny	forest drive	50°00´35.8"N, 12°44´55.4"E	19.8.2014	11	45.5	9	45.5	–
803	CZ	MAR	Rájov	grassland	50°00´28.1"N, 12°44´49.4"E	19.8.2014	9	100	0	0	–
804	CZ	MAR	Rájov	field drive	50°00´59.4"N, 12°45´00.5"E	19.8.2014	5	60	20	20	–
805	CZ	MAR	Prameny	pasture	50°03´21.6"N, 12°44´14.8"E	19.8.2014	12	0	0	100	–
806	CZ	MAR	Kladská	roadside	50°02´02.9"N, 12°41´13.7"E	20.8.2014	1	0	0	100	–

807	CZ	MAR	Podstrání	roadside	50°06′16.0"N, 12°42′05.8"E	20.8.2014	7	0	0	100	–
808	CZ	MAR	Kostelní Bříza	pasture	50°06′32.4"N, 12°38′08.2"E	20.8.2014	2	50	0	50	–
809	CZ	MAR	Studánka	pasture	50°05′17.4"N, 12°35′07.3"E	20.8.2014	15	0	0	100	–
810	CZ	MAR	Lazy	forest drive	50°04′31.4"N, 12°36′28.2"E	20.8.2014	2	0	0	100	–
811	CZ	–	Chloumek	field drive	50°22′46.2"N, 14°30′37.6"E	27.7.2014	10	0	0	100	–
812	CZ	SOB	Bořetín	arable land	49°18′26.3"N, 14°57′03.4"E	10.7.2014	30	0	0	100	–
813	CZ	SOB	Bořetín	roadside	49°18′23.7"N, 14°57′09.9"E	10.7.2014	20	0	0	100	–
814	CZ	SOB	Bořetín	arable land	49°18′47.9"N, 14°56′22.6"E	10.7.2014	18	0	0	100	–
815	CZ	SOB	Psárov	arable land	49°19′06.1"N, 14°55′18.9"E	10.7.2014	27	0	0	100	–
816	CZ	SOB	Tříklasovice	arable land	49°19′08.3"N, 14°53′58.2"E	10.7.2014	34	0	0	100	–
817	CZ	SOB	Předboř	ruderal site	49°19′04.5"N, 14°51′52.7"E	10.7.2014	4	0	0	100	–
818	CZ	SOB	Choustník	roadside	49°19′24.1"N, 14°50′23.7"E	10.7.2014	30	5	15	80	–
819	CZ	SOB	Předboř	ruderal site	49°19′21.0"N, 14°50′20.8"E	10.7.2014	15	66.7	0	33.3	–
820	CZ	SOB	Choustník	arable land	49°19′29.6"N, 14°50′28.8"E	10.7.2014	30	0	0	100	–
821	CZ	SOB	Krtov	arable land	49°20′30.4"N, 14°49′59.8"E	10.7.2014	24	62.5	0	37.5	–
822	CZ	SOB	Krtov	ruderal site	49°20′43.0"N, 14°50′04.5"E	10.7.2014	10	0	0	100	–
823	CZ	SOB	Radenín	grassland	49°22′29.4"N, 14°50′33.1"E	10.7.2014	21	0	0	100	–
824	CZ	SOB	Dlouhá Lhota	arable land	49°20′53.7"N, 14°47′26.0"E	10.7.2014	8	75	12.5	12.5	–
825	CZ	SOB	Dlouhá Lhota	roadside	49°20′28.7"N, 14°47′41.1"E	10.7.2014	29	100	0	0	–
826	CZ	SOB	Chabrovice	arable land	49°19′44.4"N, 14°47′12.9"E	10.7.2014	20	95	0	5	–
827	CZ	SOB	Cabrky	arable land	49°18′42.6"N, 14°47′20.9"E	10.7.2014	15	33	0	67	–
828	CZ	SOB	Brandlín	arable land	49°18′09.6"N, 14°46′55.5"E	10.7.2014	18	0	0	100	–
829	CZ	SOB	Košice	arable land	49°19′11.6"N, 14°45′36.7"E	10.7.2014	8	0	0	100	–
830	CZ	SOB	Doubí	roadside	49°19′19.8"N, 14°44′13.5"E	10.7.2014	20	20	0	80	–
831	CZ	SOB	Košice	roadside	49°18′50.5"N, 14°44′30.4"E	10.7.2014	10	40	0	60	–
832	CZ	SOB	Myslkovice	field drive	49°17′22.0"N, 14°44′34.2"E	10.7.2014	18	0	0	100	–
833	CZ	SOB	Sedlečko u Soběslavě	ruderal site	49°17′17.6"N, 14°44′51.2"E	10.7.2014	10	0	0	100	–
834	CZ	SOB	Sedlečko u Soběslavě	roadside	49°16′50.4"N, 14°44′17.0"E	10.7.2014	17	0	0	100	–
835	CZ	SOB	Krotějov	arable land	49°16′16.6"N, 14°46′01.1"E	10.7.2014	15	7	0	93	–
836	CZ	SOB	Kvasejovice	arable land	49°15′49.8"N, 14°46′23.8"E	10.7.2014	22	0	0	100	–
837	CZ	SOB	Kvasejovice	pond bot.	49°15′35.8"N, 14°46′52.2"E	10.7.2014	15	0	0	100	–
838	CZ	SOB	Lodhéřov	roadside	49°14′07.8"N, 14°57′13.5"E	11.7.2014	13	0	0	100	–
839	CZ	SOB	Najdek	arable land	49°13′58.4"N, 14°57′19.5"E	11.7.2014	30	3	0	97	–
840	CZ	SOB	Lodhéřov	arable land	49°12′17.6"N, 14°57′41.1"E	11.7.2014	18	0	0	100	–
841	CZ	SOB	Lodhéřov	field drive	49°12′32.2"N, 14°56′37.7"E	11.7.2014	30	97	0	3	–
842	CZ	SOB	Studnice	roadside	49°11′43.6"N, 14°57′28.1"E	11.7.2014	20	0	10	90	–
843	CZ	SOB	Studnice	fallow	49°11′23.3"N, 14°57′20.9"E	11.7.2014	25	0	0	100	–
844	CZ	SOB	Děbolín	arable land	49°10′00.1"N, 14°56′43.9"E	11.7.2014	21	0	0	100	–
845	CZ	SOB	Plasná	arable land	49°10′54.5"N, 14°53′17.9"E	11.7.2014	18	0	0	100	–

846	CZ	SOB	Klenov	arable land	49°12´16.3"N, 14°53´10.8"E	11.7.2014	25	20	0	80	–
847	CZ	SOB	Samosoly	fallow	49°13´43.6"N, 14°52´53.8"E	11.7.2014	25	0	0	100	–
848	CZ	SOB	Dírná	arable land	49°14´21.8"N, 14°51´20.4"E	11.7.2014	18	0	0	100	–
849	CZ	SOB	Dírná	arable land	49°14´11.8"N, 14°50´12.1"E	11.7.2014	15	33	0	67	–
850	CZ	SOB	Závsí	roadside	49°13´59.9"N, 14°48´46.9"E	11.7.2014	19	10	0	90	–
851	CZ	SOB	Přehořov	field drive	49°14´28.2"N, 14°45´39.9"E	11.7.2014	12	50	0	50	–
852	CZ	SOB	Mezná	pond bot.	49°15´41.0"N, 14°47´09.3"E	11.7.2014	15	0	0	100	–
853	CZ	SOB	Lipovka	arable land	49°16´59.0"N, 14°53´45.5"E	11.7.2014	8	50	0	50	–
854	CZ	SOB	Lipovka	fallow	49°16´47.2"N, 14°53´54.3"E	11.7.2014	27	93	0	7	–
855	CZ	SOB	Vícemil	arable land	49°15´34.1"N, 14°53´37.6"E	11.7.2014	15	60	0	40	–
856	CZ	SOB	Vícemil	fallow	49°15´59.9"N, 14°53´37.1"E	12.7.2014	13	0	0	100	–
857	CZ	SOB	Budislav	arable land	49°17´36.4"N, 14°51´01.7"E	12.7.2014	10	50	0	50	–
858	CZ	SOB	Chotěmice	arable land	49°16´49.6"N, 14°52´00.0"E	12.7.2014	8	100	0	0	–
859	CZ	–	Struhařov	ruderal site	49°57´23.5"N, 14°44´28.1"E	2.8.2014	11	0	0	100	–
860	CZ	RAK	Chotěšov	arable land	50°07´09.6"N, 13°29´27.1"E	1.8.2014	10	20	0	80	–
861	CZ	RAK	Tlestky	roadside	50°04´33.3"N, 13°27´51.3"E	1.8.2014	5	0	0	100	–
862	CZ	RAK	Vysoká Libyně	arable land	50°00´42.8"N, 13°28´00.6"E	1.8.2014	7	0	0	100	–
863	CZ	–	Plasy	arable land	49°55´32.1"N, 13°23´22.3"E	1.8.2014	6	33	0	67	–
864	CZ	–	Třemošná	roadside	49°48´31.0"N, 13°23´07.5"E	1.8.2014	6	0	0	100	–
865	CZ	–	Zdiby	roadside	50°10´12.0"N, 14°27´41.4"E	23.7.2014	9	0	0	100	–
866	CZ	–	Hůrky	roadside	49°02´30.4"N, 15°08´53.4"E	8.8.2014	10	0	0	100	–
867	CZ	–	Klášter u N. Bystřice	roadside	49°01´29.7"N, 15°09´08.8"E	10.8.2014	10	0	0	100	–
868	CZ	–	Šluknov	roadside	51°00´06.2"N, 14°26´02.4"E	10.8.2014	8	0	0	100	–
869	CZ	–	Valdek	roadside	50°58´56.4"N, 14°30´20.5"E	10.8.2014	6	33	0	67	–
870	CZ	–	Jestřebí	roadside	50°36´49.2"N, 14°34´15.7"E	10.8.2014	8	0	0	100	–
871	CZ	–	Ruda	arable land	49°44´30.4"N, 16°04´07.3"E	16.8.2014	6	17	0	83	–
872	CZ	RAK	Solopysky	arable land	50°15´07.1"N, 13°44´47.8"E	23.7.2014	11	100	0	0	–
873	CZ	RAK	Solopysky	grassland	50°15´01.4"N, 13°44´46.1"E	23.7.2014	11	100	0	0	–
874	CZ	RAK	Domoušice	field drive	50°13´27.2"N, 13°43´41.7"E	23.7.2014	15	21	7	72	–
875	CZ	RAK	Filipov	field drive	50°13´31.5"N, 13°43´27.1"E	23.7.2014	10	60	0	40	–
876	CZ	RAK	Lhota pod Džbánem	fallow	50°13´30.4"N, 13°43´00.6"E	23.7.2014	14	0	0	100	–
877	CZ	RAK	Kounov	pasture	50°13´44.3"N, 13°41´14.2"E	23.7.2014	4	100	0	0	–
878	CZ	RAK	Mutějovice	arable land	50°12´51.9"N, 13°42´40.4"E	23.7.2014	10	0	0	100	–
879	CZ	RAK	Perun	ruderal site	50°12´51.0"N, 13°42´40.5"E	23.7.2014	10	0	0	100	–
880	CZ	RAK	Mutějovice	arable land	50°11´58.4"N, 13°42´47.1"E	23.7.2014	10	0	0	100	–
881	CZ	RAK	Krupá	arable land	50°10´33.6"N, 13°44´25.8"E	23.7.2014	13	0	0	100	–
882	CZ	RAK	Solopysky	ruderal site	50°15´14.5"N, 13°44´49.2"E	23.7.2014	9	0	0	100	–
883	CZ	RAK	Solopysky	arable land	50°15´06.2"N, 13°44´46.9"E	23.7.2014	18	100	0	0	–
884	CZ	RAK	Domoušice-Filipov	arable land	50°13´28.1"N, 13°43´56.2"E	23.7.2014	25	40	0	60	–

885	CZ	RAK	Mutějovice	arable land	50°13´22.2"N, 13°42´05.1"E	23.7.2014	10	100	0	0	–
886	CZ	RAK	Krupá	roadside	50°10´31.8"N, 13°44´26.1"E	23.7.2014	11	0	0	100	–
887	CZ	–	Malšovice	river bank	50°44´19.9"N, 14°10´54.7"E	26.7.2014	11	0	0	100	–
888	CZ	–	Děčín	river bank	50°46´34.8"N, 14°12´23.7"E	26.7.2014	18	0	0	100	–
889	CZ	RAK	Deštnice	fallow	50°13´15.7"N, 13°35´18.7"E	29.7.2014	17	76	6	18	–
890	CZ	RAK	Nová Hospoda	roadside	50°12´55.4"N, 13°35´57.4"E	29.7.2014	11	82	0	18	–
891	CZ	RAK	Rozkoš	arable land	50°10´04.4"N, 13°38´50.7"E	29.7.2014	20	90	5	5	–
892	CZ	RAK	Rozkoš	arable land	50°10´15.5"N, 13°38´29.5"E	29.7.2014	11	82	0	18	–
893	CZ	RAK	Přílepy	arable land	50°07´58.1"N, 13°38´07.6"E	29.7.2014	6	17	0	83	–
894	CZ	RAK	Václavy	arable land	50°04´13.0"N, 13°35´26.4"E	29.7.2014	8	100	0	0	–
895	CZ	RAK	Petrovice	arable land	50°04´03.0"N, 13°38´52.1"E	29.7.2014	10	20	0	80	–
896	CZ	RAK	Pavlíkov	field drive	50°04´10.8"N, 13°45´04.8"E	29.7.2014	9	78	0	22	–
897	CZ	RAK	Všetaty	arable land	50°02´42.7"N, 13°44´10.7"E	29.7.2014	12	8	0	92	–
898	CZ	RAK	Lužná	roadside	50°06´45.6"N, 13°46´00.3"E	29.7.2014	10	0	0	100	–
899	CZ	RAK	Nová Strašecí	arable land	50°09´05.1"N, 13°51´53.2"E	29.7.2014	11	0	0	100	–
900	CZ	RAK	Ruda	ruderal site	50°08´57.1"N, 13°50´10.4"E	30.7.2014	10	30	0	70	–
901	CZ	RAK	Lužná	pasture	50°07´20.5"N, 13°47´05.2"E	30.7.2014	18	100	0	0	–
902	CZ	RAK	Lužná	arable land	50°07´03.1"N, 13°46´38.3"E	30.7.2014	13	100	0	0	–
903	CZ	RAK	Rakovník	fallow	50°07´07.3"N, 13°44´01.3"E	30.7.2014	5	80	20	0	–
904	CZ	RAK	Rakovník	grassland	50°07´08.4"N, 13°43´59.9"E	30.7.2014	6	100	0	0	–
905	CZ	RAK	Olešná	arable land	50°07´41.2"N, 13°43´38.3"E	30.7.2014	14	86	0	14	–
906	CZ	RAK	Lišany	arable land	50°09´07.1"N, 13°44´31.7"E	30.7.2014	10	0	0	100	–
907	CZ	RAK	Lišany	fallow	50°09´41.8"N, 13°44´37.5"E	30.7.2014	10	100	0	0	–
908	CZ	RAK	Lišany	fallow	50°09´31.2"N, 13°44´39.2"E	30.7.2014	14	0	0	100	–
909	CZ	RAK	Krupá	arable land	50°10´33.7"N, 13°44´25.8"E	30.7.2014	10	0	0	100	–
910	CZ	RAK	Krupá	arable land	50°10´31.3"N, 13°44´25.0"E	30.7.2014	15	0	0	100	–
911	CZ	RAK	Krupá	roadside	50°10´17.8"N, 13°43´32.6"E	30.7.2014	7	0	0	100	–
912	CZ	RAK	Krupá	arable land	50°10´17.0"N, 13°43´36.8"E	30.7.2014	10	0	0	100	–
913	CZ	RAK	Nesuchyně	arable land	50°10´27.3"N, 13°41´50.6"E	30.7.2014	14	0	0	100	–
914	CZ	RAK	Nesuchyně	arable land	50°10´25.0"N, 13°41´51.4"E	30.7.2014	13	0	0	100	–
915	CZ	RAK	Milostín	arable land	50°11´42.4"N, 13°39´50.4"E	30.7.2014	12	0	0	100	–
916	CZ	RAK	Senomaty	grassland	50°06´30.7"N, 13°40´58.3"E	31.7.2014	10	80	0	20	–
917	CZ	RAK	Senomaty	arable land	50°06´18.6"N, 13°39´58.9"E	31.7.2014	20	0	0	100	–
918	CZ	RAK	Pšovlky	arable land	50°06´29.1"N, 13°36´01.7"E	31.7.2014	16	63	0	37	–
919	CZ	RAK	Švihov	arable land	50°06´31.4"N, 13°34´38.2"E	31.7.2014	25	65	5	30	–
920	CZ	RAK	Pšovlky	arable land	50°06´29.1"N, 13°34´51.2"E	31.7.2014	14	86	0	14	–
921	CZ	RAK	Čížkov	arable land	50°07´39.4"N, 13°33´02.0"E	31.7.2014	16	0	0	100	–
922	CZ	RAK	Hředle	arable land	50°11´02.4"N, 13°44´39.4"E	1.8.2014	13	0	0	100	–
923	CZ	RAK	Mutějovice	arable land	50°11´57.9"N, 13°42´46.7"E	1.8.2014	6	0	0	100	–

924	CZ	RAK	Mutějovice	arable land	50°12´51.8"N, 13°42´40.3"E	1.8.2014	23	0	0	100	–
925	CZ	RAK	Filipov	fallow	50°13´27.2"N, 13°43´42.2"E	1.8.2014	25	8	0	91	–
926	CZ	RAK	Markvarec	field drive	50°16´45.4"N, 13°40´57.2"E	4.8.2014	12	0	0	100	–
927	CZ	RAK	Dubčany	arable land	50°17´37.8"N, 13°38´08.7"E	4.8.2014	18	0	0	100	–
928	CZ	RAK	Tasov	roadside	50°16´27.0"N, 13°35´23.3"E	4.8.2014	22	0	0	100	–
929	CZ	RAK	Kounov	arable land	50°13´32.6"N, 13°39´53.2"E	4.8.2014	21	45	10	45	–
930	CZ	RAK	Milostín	field drive	50°11´55.6"N, 13°40´12.5"E	4.8.2014	20	0	0	100	–
931	CZ	RAK	Třeboc	field drive	50°12´11.8"N, 13°44´48.7"E	4.8.2014	13	100	0	0	–
932	CZ	RAK	Janov	arable land	50°12´17.9"N, 13°38´30.5"E	4.8.2014	19	40	16	44	–
933	CZ	RAK	Mutějovice	roadside	50°11´44.8"N, 13°42´57.4"E	4.8.2014	21	0	0	100	–
934	CZ	RAK	Ročov	ruderal site	50°14´19.9"N, 13°45´32.6"E	4.8.2014	12	58	0	42	–
935	CZ	RAK	Deštnice	arable land	50°14´02.0"N, 13°35´29.6"E	4.8.2014	12	0	0	100	–
936	CZ	RAK	Lužná	pasture	50°07´20.7"N, 13°47´06.2"E	11.8.2014	5	100	0	0	–
937	CZ	RAK	Rakovník	arable land	50°07´07.3"N, 13°44´01.4"E	11.8.2014	15	100	0	0	–
938	CZ	RAK	Lišany	arable land	50°07´41.4"N, 13°43´37.8"E	11.8.2014	7	86	0	14	–
939	CZ	RAK	Lišany	fallow	50°09´31.0"N, 13°44´39.3"E	11.8.2014	10	10	0	90	–
940	CZ	RAK	Krupá	arable land	50°09´41.7"N, 13°44´37.6"E	11.8.2014	8	87	0	13	–
941	CZ	RAK	Třeboc	field drive	50°12´11.7"N, 13°44´47.6"E	11.8.2014	7	100	0	0	–
942	CZ	RAK	Filipov	field drive	50°13´31.4"N, 13°43´24.6"E	11.8.2014	5	20	0	80	–
943	CZ	RAK	Domoušice	arable land	50°13´30.3"N, 13°43´00.8"E	12.8.2014	25	10	0	15	–
944	CZ	RAK	Markvarec	field drive	50°16´45.4"N, 13°40´56.6"E	12.8.2014	5	0	0	100	–
945	CZ	RAK	Dubčany	arable land	50°17´39.0"N, 13°38´04.3"E	12.8.2014	8	0	0	100	–
946	CZ	RAK	Deštnice	arable land	50°14´03.3"N, 13°35´18.7"E	12.8.2014	5	0	0	100	–
947	CZ	RAK	Milostín	arable land	50°11´42.2"N, 13°39´49.8"E	12.8.2014	12	8	0	92	–
948	CZ	RAK	Nesuchyně	arable land	50°10´27.0"N, 13°41´49.3"E	12.8.2014	13	0	0	100	–
949	CZ	RAK	Řevničov	arable land	50°11´35.1"N, 13°47´52.0"E	13.8.2014	15	0	0	100	–
950	CZ	RAK	Konětopy	pasture	50°16´00.8"N, 13°42´30.6"E	13.8.2014	13	100	0	0	–
951	CZ	RAK	Líčkov	grassland	50°16´57.7"N, 13°37´30.1"E	13.8.2014	7	0	0	100	–
952	CZ	–	Muckov	roadside	48°44´39.6"N, 14°09´44.0"E	19.7.2014	11	0	0	100	–
953	CZ	–	Štíty	roadside	49°57´43.1"N, 16°45´57.0"E	28.6.2014	10	0	0	100	–
954	AT	–	Scheifling	roadside	47°09´19.4"N, 14°25´00.9"E	17.7.2014	10	0	0	100	–
955	AT	–	Modriach	roadside	46°58´32.7"N, 15°02´07.6"E	23.7.2014	5	0	0	100	–
956	AT	–	Tulwitz	roadside	47°16´08.6"N, 15°26´41.7"E	23.7.2014	10	0	0	100	–
957	AT	–	Hartberg	ruderal site	47°17´51.8"N, 15°57´03.1"E	23.7.2014	10	0	0	100	–
958	AT	–	Buchschachen	arable land	47°19´35.7"N, 16°07´16.9"E	27.7.2014	10	0	0	100	–
959	HU	–	Parádsasvár	grassland	47°54´11.0"N, 19°59´28.6"E	28.7.2014	3	0	0	100	–
960	HU	–	Sajópüspöki	fallow	48°16´28.9"N, 20°21´13.6"E	28.7.2014	10	0	0	100	–
961	SK	–	Hajnáčka	forest drive	48°13´29.9"N, 19°58´28.2"E	28.7.2014	10	0	0	100	–
962	SK	–	Rimavská Sobota	arable land	48°21´48.6"N, 20°00´15.5"E	28.7.2014	10	0	0	100	–

963	SK	–	Malé Straciny	roadside	48°12´23.2"N, 19°24´53.6"E	29.7.2014	5	0	0	100	–
964	SK	–	Horné Semerovce	roadside	48°08´00.3"N, 18°52´33.5"E	29.7.2014	10	0	0	100	–
965	SK	–	Kalná nad Hronom	arable land	48°11´12.6"N, 18°31´02.7"E	29.7.2014	10	0	0	100	–
966	SK	–	Malý Lapáš	arable land	48°17´53.7"N, 18°10´11.4"E	29.7.2014	10	0	0	100	–
967	CZ	–	Rojice	pond bot.	49°21´01.0"N, 13°56´30.2"E	7.8.2014	3	0	0	100	–
968	CZ	–	Blatná	pond bot.	49°26´00.2"N, 13°53´50.0"E	7.8.2014	5	0	0	100	–
969	CZ	–	Blatná	river bank	49°25´17.0"N, 13°52´47.0"E	7.8.2014	2	0	0	100	–
970	CZ	–	Češnovice	arable land	49°01´51.5"N, 14°22´04.1"E	6.8.2014	2	0	0	100	–
971	CZ	–	Břehov	arable land	49°01´00.4"N, 14°21´14.6"E	6.8.2014	11	0	0	100	–
972	CZ	–	Hrabišín	grassland	49°54´16.6"N, 17°02´35.4"E	6.8.2014	10	90	0	10	–
973	CZ	–	Bystřice p. Lopen.	roadside	48°57´54.4"N, 17°46´09.3"E	9.8.2014	5	0	0	100	–
974	CZ	–	Bílavsko	arable land	49°23´23.7"N, 17°38´07.8"E	9.8.2014	14	0	0	100	–
975	PL	–	Rusocin	arable land	50°30´09.8"N, 17°23´38.6"E	6.8.2014	10	100	0	0	–
976	CZ	–	Ostrov u Macochy	ruderal site	49°23´08.5"N, 16°46´16.8"E	9.8.2014	10	0	0	100	–
977	CZ	–	Rudice	ruderal site	49°19´54.2"N, 16°42´59.5"E	8.8.2014	10	0	0	100	–
978	CZ	–	Niva	roadside	49°26´14.8"N, 16°51´38.9"E	9.8.2014	10	90	0	10	–
979	CZ	–	Prostějov	arable land	49°28´19.7"N, 17°03´50.0"E	9.8.2014	10	0	0	100	–
980	CZ	–	Lomnice	forest drive	49°51´25.2"N, 17°24´35.3"E	12.8.2014	5	0	0	100	–
981	CZ	–	Dolany	roadside	49°39´14.8"N, 17°18´35.3"E	12.8.2014	10	0	0	100	–
982	CZ	–	Lipina	arable land	49°45´31.3"N, 17°20´28.3"E	12.8.2014	10	73	9	18	–
983	CZ	–	Stříbrné Hory	pasture	49°56´06.3"N, 17°12´09.9"E	11.8.2014	20	0	0	100	–
984	SK	–	Prietrž	arable land	48°40´22.0"N, 17°27´05.0"E	23.8.2014	20	0	0	100	–
985	DE	–	Großseeham	ruderal site	47°51´41.2"N, 11°51´43.0"E	18.8.2014	2	0	0	100	–
986	DE	–	Kranzberg	ruderal site	48°23´19.7"N, 11°35´55.0"E	18.8.2014	11	0	0	100	–
987	DE	–	Regensburg	ruderal site	48°59´32.9"N, 12°05´38.0"E	18.8.2014	16	12.5	0	87.5	–
988	DE	–	Damelsdorf	roadside	49°31´12.8"N, 12°09´58.9"E	18.8.2014	11	0	0	100	–
989	CZ	–	Seníky	roadside	50°08´44.2"N, 12°20´20.3"E	14.8.2014	3	0	0	100	–
990	CZ	MAR	Nahý Újezdec	ruderal site	49°50´11.9"N, 12°42´17.9"E	14.8.2014	1	0	0	100	–
991	CZ	MAR	Brod nad Tichou	pond bot.	49°50´22.4"N, 12°42´49.9"E	14.8.2014	9	11	0	89	–
992	CZ	–	Nerestce	arable land	49°29´56.4"N, 14°04´22.7"E	19.8.2014	11	0	0	100	–
993	CZ	–	Rojice	pond bot.	49°21´01.2"N, 13°56´56.9"E	19.8.2014	13	0	0	100	–
994	CZ	–	Peč	roadside	49°03´38.6"N, 15°23´28.6"E	20.8.2014	5	0	0	100	–
995	CZ	–	Třešť	pond bot.	49°16´55.1"N, 15°32´11.1"E	20.8.2014	7	0	0	100	–
996	CZ	–	Boskovice	arable land	49°28´24.3"N, 16°38´06.5"E	16.8.2014	15	0	0	100	–
997	DE	–	Haslach	arable land	48°50´39.4"N, 13°29´38.2"E	27.8.2014	7	0	0	100	–
998	DE	–	Allhartsmais	roadside	48°51´03.7"N, 13°12´40.9"E	27.8.2014	3	0	0	100	–
999	DE	–	Schwarzach	arable land	48°46´58.7"N, 13°02´30.2"E	27.8.2014	13	0	0	100	–
1000	DE	–	Haidlfing	arable land	48°43´27.9"N, 12°43´23.9"E	27.8.2014	7	0	0	100	–
1001	DE	–	Unterhollerau	arable land	48°40´06.1"N, 12°27´48.9"E	27.8.2014	6	0	0	100	–

1002	DE	–	Pfettrach	arable land	48°34´40.9"N, 12°04´22.4"E	27.8.2014	19	0	0	100	–
1003	DE	–	Pfeffenhausen	arable land	48°40´12.7"N, 11°57´11.6"E	27.8.2014	2	0	0	100	–
1004	DE	–	Siegenburg	arable land	48°45´25.6"N, 11°51´20.0"E	27.8.2014	11	0	0	100	–
1005	DE	–	Sinzing	arable land	48°58´54.4"N, 12°02´05.3"E	27.8.2014	3	0	0	100	–
1006	DE	–	Essing	arable land	48°55´50.8"N, 11°47´44.5"E	27.8.2014	6	0	0	100	–
1007	DE	–	Mühlbach	ruderal site	49°01´45.8"N, 11°36´42.8"E	28.8.2014	14	0	0	100	–
1008	DE	–	Unteremmendorf	arable land	48°59´50.6"N, 11°26´08.8"E	28.8.2014	7	0	0	100	–
1009	DE	–	Sindersdorf	arable land	49°09´43.0"N, 11°16´08.7"E	28.8.2014	10	0	0	100	–
1010	DE	–	Tennenlohe	arable land	49°32´35.0"N, 11°01´58.7"E	28.8.2014	12	0	0	100	–
1011	DE	–	Dechsendorf	arable land	49°37´22.1"N, 10°56´51.9"E	28.8.2014	8	100	0	0	–
1012	DE	–	Schirnsdorf	arable land	49°44´32.6"N, 10°47´11.4"E	28.8.2014	1	0	0	100	–
1013	DE	–	Füttersee	arable land	49°46´41.4"N, 10°29´24.3"E	28.8.2014	10	0	0	100	–
1014	DE	–	Karbach	arable land	49°54´09.5"N, 10°33´31.1"E	28.8.2014	3	0	0	100	–
1015	DE	–	Eltmann	arable land	49°58´31.2"N, 10°40´11.8"E	28.8.2014	2	0	0	100	–
1016	DE	–	Burgellern	ruderal site	49°59´12.9"N, 11°03´09.2"E	28.8.2014	9	0	0	100	–
1017	DE	–	Wotzendorf	arable land	49°59´32.3"N, 11°13´21.9"E	28.8.2014	15	0	0	100	–
1018	DE	–	Wadendorf	arable land	49°53´45.8"N, 11°19´59.2"E	28.8.2014	11	0	0	100	–
1019	DE	–	Gössweinstein	roadside	49°45´49.9"N, 11°20´36.7"E	29.8.2014	12	50	0	50	–
1020	DE	–	Wannberg	arable land	49°44´09.3"N, 11°27´47.2"E	29.8.2014	9	0	44	56	–
1021	DE	–	Neunkirchen a. M.	arable land	49°54´58.8"N, 11°38´57.3"E	29.8.2014	11	100	0	0	–
1022	DE	–	Lettenhof	arable land	49°52´20.6"N, 11°48´52.5"E	29.8.2014	12	100	0	0	–
1023	DE	–	Frauenberg Grötsch.	arable land	49°51´19.9"N, 12°01´52.6"E	29.8.2014	1	100	0	0	–
1024	DE	–	Steinbach	arable land	49°49´04.2"N, 12°04´40.3"E	29.8.2014	7	0	0	100	–
1025	DE	–	Neustadt a. d. W.	ruderal site	49°43´53.0"N, 12°08´04.4"E	29.8.2014	10	0	0	100	–
1026	DE	–	Unterwildenau	arable land	49°35´49.0"N, 12°08´13.3"E	29.8.2014	12	0	0	100	–
1027	DE	–	Nabburg	arable land	49°27´18.4"N, 12°12´09.7"E	29.8.2014	9	0	0	100	–
1028	DE	–	Klardorf	arable land	49°16´38.5"N, 12°08´16.5"E	29.8.2014	5	0	0	100	–
1029	DE	–	Rosenhof	arable land	48°59´21.5"N, 12°14´36.7"E	29.8.2014	11	0	0	100	–
1030	DE	–	Sollach	roadside	48°55´47.6"N, 12°42´27.4"E	29.8.2014	3	0	0	100	–
1031	DE	–	Grubhof	roadside	48°41´15.8"N, 13°25´35.5"E	29.8.2014	5	0	0	100	–
1032	DE	–	Linden	roadside	48°49´05.8"N, 13°34´08.1"E	29.8.2014	1	0	0	100	–
1033	CZ	–	Kopanina	ruderal site	50°31´02.9"N, 15°13´47.2"E	31.8.2014	12	92	0	8	–
1034	CZ	–	Bílé Poličany	roadside	50°23´39.8"N, 15°43´02.2"E	31.8.2014	3	0	0	100	–
1035	AT	–	Schindlau	arable land	48°39´59.3"N, 13°56´35.3"E	3.8.2014	10	0	0	100	–
1036	AT	–	Schindlau	ruderal site	48°39´59.1"N, 13°56´35.2"E	3.8.2014	10	0	0	100	–
1037	DE	–	Großdittmannsdorf	roadside	51°11´31.3"N, 13°46´23.1"E	18.9.2014	3	0	0	100	–
1038	DE	–	Seefeld	arable land	52°36´44.5"N, 13°39´11.3"E	18.9.2014	6	66.7	0	33.3	–
1039	DE	–	Werneuchen	ruderal site	52°38´11.1"N, 13°44´40.4"E	18.9.2014	5	0	0	100	–
1040	DE	–	Cedynia	river bank	52°53´22.1"N, 14°09´44.5"E	19.9.2014	8	0	0	100	–

1041	PL	–	Osinów Dolny	grassland	52°51´35.5"N, 14°09´20.5"E	19.9.2014	7	0	0	100	–
1042	PL	–	Bielinek	ruderal site	52°56´49.7"N, 14°08´46.5"E	19.9.2014	8	0	0	100	–
1043	PL	–	Piasek	ruderal site	52°58´07.1"N, 14°10´25.1"E	19.9.2014	6	0	0	100	–
1044	PL	–	Piasek	ruderal site	52°58´38.6"N, 14°12´09.0"E	19.9.2014	1	0	0	100	–
1045	PL	–	Wielka Kepa	arable land	53°09´41.7"N, 18°12´33.3"E	22.9.2014	6	0	0	100	–
1046	PL	–	Toruń	arable land	53°00´40.4"N, 18°40´48.5"E	22.9.2014	9	0	0	100	–
1047	PL	–	Zlotoria	roadside	52°59´07.7"N, 18°42´15.8"E	22.9.2014	4	0	0	100	–
1048	PL	–	Czerniewice	ruderal site	52°59´09.4"N, 18°41´58.6"E	22.9.2014	2	0	0	100	–
1049	PL	–	Ciechocinek	river bank	52°53´28.7"N, 18°48´52.1"E	22.9.2014	1	0	0	100	–
1050	CZ	SOB	Mezná	arable land	49°15´32.3"N, 14°48´23.4"E	1.8.2014	22	0	0	100	–
1051	CZ	SOB	Chotěmice	arable land	49°16´24.6"N, 14°52´04.9"E	1.8.2014	15	100	0	0	–
1052	CZ	SOB	Chotěmice	arable land	49°16´11.8"N, 14°52´38.2"E	1.8.2014	25	88	0	12	neo4x (3.42, CV = 2.30%)
1053	CZ	SOB	Vícemil	arable land	49°15´42.3"N, 14°52´52.5"E	1.8.2014	31	100	0	0	–
1054	CZ	SOB	Červená Lhota	pond bot.	49°15´24.8"N, 14°53´02.9"E	1.8.2014	25	16	4	80	–
1055	CZ	SOB	Turovec	pond bot.	49°22´36.6"N, 14°46´13.0"E	1.8.2014	8	0	0	100	–
1056	CZ	SOB	Třebějice	grassland	49°14´35.0"N, 14°49´02.6"E	1.8.2014	7	14	0	86	–
1057	CZ	SOB	Dírná	arable land	49°14´12.2"N, 14°50´12.6"E	1.8.2014	28	0	0	100	6x (5.81, CV = 1.40%)
1058	CZ	SOB	Vícemil	roadside	49°15´16.8"N, 14°53´43.7"E	1.8.2014	12	8	0	92	–
1059	CZ	SOB	Deštná	grassland	49°15´23.9"N, 14°56´05.9"E	2.8.2014	7	100	0	0	–
1060	CZ	SOB	Deštná	roadside	49°15´43.7"N, 14°55´59.2"E	2.8.2014	30	60	7	33	–
1061	CZ	SOB	Deštná	arable land	49°15´52.0"N, 14°55´41.6"E	2.8.2014	8	100	0	0	–
1062	CZ	SOB	Deštná	ruderal site	49°15´21.2"N, 14°54´53.6"E	2.8.2014	12	50	0	50	–
1063	CZ	SOB	Jižná	pond bot.	49°15´08.2"N, 14°54´34.3"E	2.8.2014	15	66	0	33	–
1064	CZ	RAK	Olešná	arable land	50°07´30.0"N, 13°40´06.7"E	20.9.2014	10	0	0	100	–
1065	CZ	RAK	Kněževes	arable land	50°08´13.5"N, 13°39´02.9"E	20.9.2014	10	90	10	0	–
1066	CZ	RAK	Přílepy	arable land	50°07´45.3"N, 13°38´05.6"E	20.9.2014	5	100	0	0	–
1067	CZ	RAK	Kolešovice	arable land	50°08´28.9"N, 13°37´16.9"E	20.9.2014	11	0	0	100	–
1068	CZ	RAK	Kněževes	roadside	50°09´46.5"N, 13°38´23.0"E	20.9.2014	6	17	0	83	–
1069	CZ	RAK	Nový Dvůr	arable land	50°10´03.5"N, 13°39´28.5"E	20.9.2014	8	0	0	100	–
1070	CZ	RAK	Lubná	ruderal site	50°04´45.3"N, 13°42´49.1"E	20.9.2014	6	0	0	100	–
1071	CZ	RAK	Senec	arable land	50°03´42.8"N, 13°42´15.8"E	20.9.2014	3	0	0	100	–
1072	CZ	RAK	Hvozd	field drive	50°02´52.4"N, 13°41´13.5"E	20.9.2014	7	0	0	100	–
1073	CZ	RAK	Příčina	arable land	50°03´43.2"N, 13°40´15.5"E	20.9.2014	5	40	20	40	–
1074	CZ	RAK	Hostokryje	arable land	50°04´44.4"N, 13°40´33.3"E	20.9.2014	12	52	40	8	–
1075	CZ	RAK	Hostokryje	field drive	50°05´24.0"N, 13°40´30.3"E	21.9.2014	7	0	0	100	–
1076	CZ	RAK	Rakovník	arable land	50°05´42.6"N, 13°41´03.8"E	21.9.2014	8	0	0	100	–
1077	CZ	RAK	Keblany	arable land	50°08´39.0"N, 13°35´23.4"E	21.9.2014	10	0	0	100	–

1078	CZ	RAK	Hořovičky	roadside	50°09´27.3"N, 13°32´55.7"E	21.9.2014	4	0	0	100	–
1079	CZ	RAK	Vrbice	arable land	50°10´14.4"N, 13°31´36.5"E	21.9.2014	10	0	0	100	–
1080	CZ	RAK	Děkov	arable land	50°10´35.2"N, 13°33´26.7"E	21.9.2014	7	0	0	100	–
1081	CZ	RAK	Velká Černoc	arable land	50°11´21.5"N, 13°35´16.7"E	21.9.2014	6	0	0	100	–
1082	CZ	RAK	Svojetín	roadside	50°11´42.2"N, 13°36´26.2"E	22.9.2014	7	0	14	86	–
1083	CZ	RAK	Senkov	arable land	50°17´46.2"N, 13°46´50.9"E	22.9.2014	10	100	0	0	–
1084	CZ	RAK	Kozojedy	arable land	50°15´34.3"N, 13°49´12.8"E	22.9.2014	4	0	0	100	–
1085	CZ	RAK	Smolnice	roadside	50°18´31.4"N, 13°50´42.3"E	22.9.2014	6	0	0	100	–
1086	CZ	RAK	Kroučová	roadside	50°12´12.9"N, 13°48´12.2"E	22.9.2014	2	50	0	50	–
1087	CZ	–	Ostrava	ruderal site	49°51´00.3"N, 18°16´10.6"E	25.9.2014	6	0	0	100	–
1088	PL	–	Kraków	ruderal site	50°04´08.8"N, 19°54´02.5"E	26.9.2014	9	0	0	100	–
1089	CZ	–	Vrbice	roadside	49°52´38.8"N, 18°18´37.8"E	27.9.2014	10	20	0	80	–
1090	CZ	–	Petřkovice	ruderal site	49°52´03.0"N, 18°15´52.1"E	27.9.2014	3	0	0	100	–
1091	CZ	RAK	Rakovník	arable land	50°05´04.5"N, 13°43´24.3"E	16.9.2014	25	96	0	4	–
1092	CZ	RAK	Rakovník	arable land	50°05´16.9"N, 13°43´31.9"E	16.9.2014	12	0	0	100	–
1093	CZ	RAK	Pavlíkov	roadside	50°03´53.8"N, 13°44´35.1"E	16.9.2014	1	0	0	100	–
1094	CZ	RAK	Lubná	arable land	50°04´22.7"N, 13°41´09.8"E	16.9.2014	14	71	0	29	3x-4x (2.68, CV = 1.14%)
1095	CZ	RAK	Řeřichy	arable land	50°05´07.4"N, 13°35´00.6"E	16.9.2014	13	38	24	38	–
1096	CZ	RAK	Zderaz	arable land	50°08´21.3"N, 13°35´12.8"E	16.9.2014	11	0	0	100	–
1097	CZ	RAK	Na Kokrdech	arable land	50°07´07.2"N, 13°44´00.6"E	16.9.2014	25	92	0	8	–
1098	CZ	RAK	Svojetín	arable land	50°11´55.9"N, 13°37´32.3"E	16.9.2014	6	83	17	0	–
1099	CZ	RAK	Veclov	roadside	50°11´01.6"N, 13°37´52.5"E	16.9.2014	5	0	20	80	–
1100	CZ	RAK	Deštnice	arable land	50°13´22.6"N, 13°35´07.2"E	17.9.2014	11	100	0	0	–
1101	CZ	RAK	Deštnice	arable land	50°13´22.9"N, 13°36´20.2"E	17.9.2014	10	20	20	60	–
1102	CZ	RAK	Deštnice	arable land	50°13´52.1"N, 13°36´11.5"E	17.9.2014	1	0	0	100	–
1103	CZ	RAK	Lhota	roadside	50°15´30.3"N, 13°35´18.5"E	17.9.2014	4	0	0	100	–
1104	CZ	RAK	Nečemice	grassland	50°14´52.8"N, 13°37´22.9"E	17.9.2014	2	0	0	100	–
1105	CZ	RAK	Nečemice	fallow	50°14´45.2"N, 13°38´52.3"E	17.9.2014	11	0	0	100	–
1106	FR	–	Heining-L.-Bouzon.	arable land	49°17´52.6"N, 06°35´26.5"E	14.9.2014	12	84	8	8	–
1107	DE	–	Grombach	roadside	49°13´19.0"N, 08°58´12.5"E	14.9.2014	14	0	0	100	–
1108	CZ	–	Královec	railway	50°40´25.8"N, 15°58´16.7"E	8.9.2014	6	0	0	100	–
1109	CZ	–	Pilníkov	railway	50°31´59.7"N, 15°48´40.3"E	8.9.2014	6	0	0	100	–
1110	CZ	–	Milíkovice	pond bot.	48°51´57.6"N, 14°26´12.1"E	3.9.2014	3	0	0	100	–
1111	CZ	–	Milíkovice	pond bot.	48°52´27.9"N, 14°26´46.5"E	3.9.2014	9	0	0	100	–
1112	CZ	–	Tržek	pond bot.	49°53´22.5"N, 16°15´38.4"E	7.10.2014	5	80	0	20	–
1113	CZ	–	Litomyšl	roadside	49°51´35.9"N, 16°20´49.4"E	7.10.2014	5	0	0	100	–
1114	CZ	–	Moravská Třebová	pond bot.	49°44´44.2"N, 16°39´17.7"E	7.10.2014	9	100	0	0	–
1115	CZ	–	Rapotín	roadside	49°59´37.9"N, 17°00´33.0"E	7.10.2014	9	11	0	89	–

1116	CZ	–	Lipina	grassland	49°45´29.5"N, 17°20´29.0"E	8.10.2014	5	0	0	100	–
1117	CZ	–	Bílčice	roadside	49°51´44.7"N, 17°33´36.8"E	8.10.2014	6	83	0	17	–
1118	CZ	–	Leskovec n. Morav.	pond bot.	49°55´01.0"N, 17°33´19.0"E	8.10.2014	5	100	0	0	–
1119	CZ	–	Deštné	roadside	49°53´39.1"N, 17°39´57.4"E	8.10.2014	5	40	0	60	–
1120	CZ	–	Frýdlant n. Ostrav.	roadside	49°35´03.3"N, 18°21´09.8"E	8.10.2014	2	0	0	100	–
1121	CZ	–	Kokory	ruderal site	49°30´02.2"N, 17°22´02.4"E	9.10.2014	6	0	0	100	–
1122	CZ	–	Tržek	roadside	49°53´26.3"N, 16°15´32.2"E	7.10.2014	7	0	0	100	–
1123	CZ	–	Rudoltice	roadside	49°59´15.4"N, 17°06´16.9"E	7.10.2014	11	100	0	0	–
1124	AT	–	Rainbach i. Mühlkr.	ruderal site	48°33´12.7"N, 14°28´57.6"E	1.11.2014	2	0	0	100	–
1125	AT	–	Freistadt	ruderal site	48°31´30.0"N, 14°30´17.6"E	1.11.2014	9	0	0	100	–
1126	AT	–	Mauthausen	arable land	48°14´51.4"N, 14°32´13.5"E	1.11.2014	8	0	0	100	–
1127	AT	–	Wallsee	ruderal site	48°09´52.9"N, 14°43´27.2"E	1.11.2014	5	0	0	100	–
1128	AT	–	Stephanshart	arable land	48°10´08.9"N, 14°47´11.7"E	1.11.2014	10	100	0	0	–
1129	AT	–	Ysper	roadside	48°18´03.9"N, 15°04´16.9"E	1.11.2014	1	0	0	100	–
1130	AT	–	Bergern	ruderal site	48°19´58.4"N, 15°12´01.6"E	1.11.2014	1	0	0	100	–
1131	AT	–	Eck	ruderal site	48°23´08.9"N, 15°14´00.4"E	2.11.2014	3	0	0	100	–
1132	AT	–	Pleßberg	arable land	48°24´08.4"N, 15°14´13.7"E	2.11.2014	6	0	0	100	–
1133	AT	–	Armschlag	roadside	48°27´00.7"N, 15°13´20.5"E	2.11.2014	4	0	0	100	–
1134	AT	–	Grainbrunn	arable land	48°29´00.3"N, 15°15´40.2"E	2.11.2014	10	0	0	100	–
1135	AT	–	Marbach im Felde	roadside	48°32´33.6"N, 15°21´04.0"E	2.11.2014	2	0	0	100	–
1136	AT	–	Mitterreith	arable land	48°36´36.5"N, 15°15´47.4"E	2.11.2014	2	0	0	100	–
1137	AT	–	Rudmanns	ruderal site	48°36´21.1"N, 15°13´12.0"E	2.11.2014	3	0	0	100	–
1138	AT	–	Zwettl Stift	pond bot.	48°36´48.1"N, 15°13´15.5"E	2.11.2014	4	0	0	100	–
1139	AT	–	Kleehof	arable land	48°35´00.2"N, 15°13´44.0"E	2.11.2014	4	0	0	100	–
1140	DE	–	Börnersdorf	ruderal site	50°49´20.9"N, 13°53´13.1"E	29.10.2014	6	80	0	20	–
1141	DE	–	Rundteil	roadside	50°56´51.7"N, 13°42´02.6"E	29.10.2014	9	0	0	100	–
1142	DE	–	Dresden	arable land	51°02´00.9"N, 13°40´18.6"E	29.10.2014	8	0	0	100	–
1143	DE	–	Kreischa	roadside	50°56´44.7"N, 13°44´33.9"E	29.10.2014	10	0	0	100	–
1144	CZ	–	Královec	roadside	50°40´16.4"N, 15°57´48.5"E	2.11.2014	3	100	0	0	–
1145	PL	–	Lubawka	roadside	50°43´02.9"N, 16°00´13.4"E	2.11.2014	2	100	0	0	–
1146	PL	–	Paprotki	roadside	50°43´25.1"N, 15°56´21.0"E	2.11.2014	4	75	0	25	–
1147	PL	–	Sobieszów	roadside	50°50´35.4"N, 15°38´35.9"E	2.11.2014	5	100	0	0	–
1148	PL	–	Pobiedna	roadside	50°55´50.0"N, 15°17´13.3"E	2.11.2014	5	0	0	100	–
1149	PL	–	Janice	roadside	50°58´03.7"N, 15°31´40.0"E	2.11.2014	5	100	0	0	–
1150	CZ	–	Vernéřovice	roadside	50°36´42.0"N, 16°13´16.1"E	3.11.2014	5	0	0	100	–
1151	PL	–	Olszyniec	roadside	50°43´19.3"N, 16°22´16.0"E	3.11.2014	2	0	0	100	–
1152	PL	–	Przerzeczyn-Zdrój	roadside	50°41´06.2"N, 16°48´51.9"E	3.11.2014	7	57	0	43	–
1153	PL	–	Cieplowody	roadside	50°39´18.0"N, 16°54´03.8"E	3.11.2014	9	78	11	11	–
1154	PL	–	Zablocie	roadside	50°19´45.5"N, 16°39´41.5"E	3.11.2014	4	0	0	100	–

1155	PL	–	Grodków	roadside	50°41´37.7"N, 17°21´47.2"E	4.11.2014	7	0	0	100	–
1156	PL	–	Mikowice	roadside	51°01´46.8"N, 17°33´28.0"E	4.11.2014	4	0	25	75	–
1157	PL	–	Czeszów	roadside	51°22´50.1"N, 17°15´14.5"E	4.11.2014	5	0	0	100	–
1158	PL	–	Spalona	roadside	51°14´40.9"N, 16°17´15.8"E	4.11.2014	6	0	0	100	–
1159	CZ	–	Čihák	roadside	50°07´52.1"N, 16°35´58.8"E	26.10.2014	12	8	0	92	–
1160	CZ	–	Křesanov	ruderal site	49°04´13.7"N, 13°44´44.0"E	7.11.2014	2	0	0	100	–
1161	CZ	–	Svatá Máří	roadside	49°03´45.3"N, 13°49´45.2"E	7.11.2014	4	0	0	100	–
1162	PL	–	Ksawerów	roadside	51°02´13.2"N, 15°02´55.5"E	18.11.2014	4	100	0	0	–
1163	PL	–	Poźrzadlo	roadside	52°17´52.7"N, 15°14´56.5"E	18.11.2014	3	0	0	100	–
1164	PL	–	Rozlogi	roadside	52°15´04.7"N, 15°29´20.6"E	18.11.2014	6	0	0	100	–
1165	PL	–	Zielona Góra	roadside	51°57´01.8"N, 15°33´06.3"E	18.11.2014	4	0	0	100	–
1166	PL	–	Zimna Brzeźnica	roadside	51°39´36.0"N, 15°49´19.0"E	18.11.2014	6	83	0	17	–
1167	PL	–	Kochlice	roadside	51°17´11.1"N, 16°09´07.8"E	18.11.2014	8	0	0	100	–
1168	PL	–	Skorochów	roadside	50°28´39.4"N, 17°16´41.0"E	18.11.2014	5	100	0	0	–
1169	PL	–	Piorunkowice	ruderal site	50°23´55.4"N, 17°30´27.8"E	18.11.2014	3	0	33	67	–
1170	PL	–	Rzepcze	arable land	50°22´29.8"N, 17°52´16.6"E	18.11.2014	6	0	0	100	–
1171	PL	–	Chrzowice	roadside	50°36´49.1"N, 17°55´43.6"E	18.11.2014	7	43	0	57	–
1172	PL	–	Malerzowice Wielkie	arable land	50°35´15.7"N, 17°29´19.9"E	18.11.2014	6	100	0	0	–
1173	CZ	–	Rozkoš	field drive	49°01´50.7"N, 15°59´28.8"E	18.11.2014	4	0	0	100	–
1174	CZ	–	Trutnov	field drive	50°34´28.3"N, 15°52´37.2"E	18.11.2014	2	0	0	100	–
1175	CZ	–	Levínská Olešnice	arable land	50°31´29.5"N, 15°31´54.3"E	15.10.2014	3	100	0	0	–
1176	CZ	–	Vrchlabí	railway	50°37´06.4"N, 15°37´20.6"E	15.10.2014	1	0	0	100	–
1177	CZ	SOB	Krtov	ruderal site	49°20´39.2"N, 14°50´01.7"E	10.10.2014	11	0	0	100	–
1178	CZ	–	Lešany	field drive	50°15´10.6"N, 14°17´06.6"E	12.9.2014	11	0	0	100	–
1179	CZ	–	Červená Řečice	arable land	49°30´12.0"N, 15°10´46.2"E	25.8.2014	7	43	0	57	–
1180	CZ	–	Krátká Ves	roadside	49°37´15.9"N, 15°40´26.5"E	25.8.2014	5	20	0	80	–
1181	CZ	–	Znětínek	pond bot.	49°27´33.1"N, 15°55´39.9"E	25.8.2014	7	29	0	71	–
1182	CZ	–	Jílové	grassland	50°39´18.8"N, 15°06´05.1"E	25.8.2014	7	29	29	42	neo4x (3.26, CV = 1.30%)
1183	CZ	–	Rakvice	fallow	48°51´44.9"N, 16°50´16.9"E	25.8.2014	12	0	0	100	–
1184	CZ	–	Škrdlovice	ruderal site	49°38´13.3"N, 15°55´14.9"E	21.8.2014	7	0	0	100	–
1185	CZ	–	Jablonné v Podještědí	roadside	50°46´12.3"N, 14°44´11.3"E	22.8.2014	3	0	67	33	–
1186	CZ	–	Šedivec	fallow	50°03´16.2"N, 16°30´49.1"E	30.7.2014	4	100	0	0	–
1187	CZ	–	Letohrad	railway	50°02´03.3"N, 16°30´07.7"E	30.7.2014	7	0	0	100	–
1188	CZ	–	Lohenice	arable land	50°02´40.7"N, 15°36´06.4"E	30.7.2014	8	25	0	75	–
1189	CZ	–	Nekoř	arable land	50°03´45.8"N, 16°31´31.6"E	30.7.2014	11	100	0	0	–
1190	CZ	–	Svitavy	arable land	49°45´37.7"N, 16°30´33.5"E	9.11.2014	10	0	0	100	–
1191	CZ	–	Rychnov	arable land	49°46´05.1"N, 16°03´49.0"E	9.11.2014	10	0	0	100	–
1192	CZ	–	Oudoleň	arable land	49°40´31.6"N, 15°44´51.4"E	9.11.2014	10	0	0	100	–

1193	CZ	–	Ježkovice	arable land	49°18′24.5"N, 16°53′04.8"E	8.11.2014	10	0	0	100	–
1194	CZ	–	Výšice	arable land	49°27′02.4"N, 13°59′55.1"E	2.11.2014	10	0	0	100	–
1195	CZ	–	Zbraň	ruderal site	50°08′29.8"N, 15°21′13.6"E	26.10.2014	10	0	0	100	–
1196	CZ	–	Slavkovice	arable land	49°28′55.7"N, 14°01′43.3"E	25.10.2014	10	0	0	100	–
1197	CZ	SOB	Na Samotách	arable land	49°10′33.8"N, 14°58′43.3"E	12.10.2014	10	0	0	100	–
1198	CZ	RAK	Ruda	arable land	50°06′46.0"N, 13°52′22.0"E	28.9.2014	10	100	0	0	–
1199	CZ	RAK	Roztoky	ruderal site	50°01′23.3"N, 13°50′49.7"E	27.9.2014	6	50	0	50	–
1200	CZ	–	Klení	grassland	48°45′28.8"N, 14°37′31.7"E	28.9.2014	10	0	0	100	–
1201	CZ	–	Chodeč	arable land	48°48′48.8"N, 14°27′35.4"E	28.9.2014	10	0	0	100	–
1202	CZ	–	Trhové Sviny	roadside	48°50′07.0"N, 14°38′19.6"E	28.9.2014	5	0	0	100	–
1203	CZ	–	Hradiště	roadside	48°44′05.3"N, 14°32′19.2"E	28.9.2014	10	0	0	100	–
1204	CZ	–	Děkanské Skaliny	ruderal site	48°44′25.5"N, 14°33′26.9"E	28.9.2014	9	0	0	100	–
1205	CZ	–	Nové Dobrkovice	roadside	48°48′57.0"N, 14°17′05.3"E	22.9.2014	10	0	0	100	–
1206	CZ	–	Kladenské Rovné	roadside	48°47′31.3"N, 14°13′53.3"E	22.9.2014	10	0	0	100	–
1207	HU	–	Szigetújfalu	ruderal site	47°14′01.9"N, 18°54′49.2"E	30.8.2015	5	0	0	100	–
1208	HU	–	Balatonfuzfo	grassland	47°04′23.0"N, 18°02′35.6"E	28.8.2015	8	0	0	100	–
1209	HU	–	Cegléd	grassland	47°11′24.6"N, 19°52′37.9"E	30.8.2015	5	0	0	100	–

[*] AT – Austria, CH – Switzerland, CZ – Czech Republic, DE – Germany, FR – France, HU – Hungary, PL – Poland, SK – Slovakia.

[†] Populations that were part of a medium-scale ploidy screening are assigned to one of the three study regions (MAR, RAK, SOB).

[‡] Number of individuals analyzed for ploidy level using FCM.

[#] Minority cytotypes discovered in populations based on their unusual relative genome size. A presumable cytotype identity is followed by the details of FCM analysis (relative genome size, a coefficient of variation). Neotetraploid mutants (neo4x) were discerned from the long-established tetraploids (4x). Aneuploids were assigned into three arbitrarily-set classes delimited by the closest lower and higher euploid cytotype: 2x–3x, 3x–4x, and 4x–5x.

Table S2. Relative genome size of 1,263 seedlings germinated from open-pollinated achenes collected in one diploid, one tetraploid and five mixed-ploidy populations of *Tripleurospermum inodorum*. Chromosome counts made on 16 individuals were used for inferring cytotypes of the offspring.

Population of achene origin		**FCM analysis of seedling**			**Chromosome count**
number[*]	ploidy composition	relative genome size[†]	CV [%][‡]	estimated cytotype	
858	2x	1.683	1.65	2x	–
858	2x	1.683	1.65	2x	–
858	2x	1.683	1.65	2x	–
858	2x	1.683	1.65	2x	–
858	2x	1.683	2.10	2x	–
858	2x	1.683	2.10	2x	–
858	2x	1.683	2.10	2x	–
858	2x	1.683	2.10	2x	–
858	2x	1.683	2.10	2x	–
858	2x	1.684	1.40	2x	–
858	2x	1.684	1.40	2x	–
858	2x	1.684	1.40	2x	–
858	2x	1.684	1.40	2x	–
858	2x	1.684	2.62	2x	–
858	2x	1.684	2.62	2x	–
858	2x	1.684	2.62	2x	–
858	2x	1.684	2.62	2x	–
858	2x	1.684	2.32	2x	–
858	2x	1.684	2.32	2x	–
858	2x	1.684	2.32	2x	–
858	2x	1.684	2.32	2x	–
858	2x	1.684	2.32	2x	–
858	2x	1.687	2.32	2x	–
858	2x	1.687	2.32	2x	–
858	2x	1.687	2.32	2x	–
858	2x	1.687	2.32	2x	–
858	2x	1.687	2.32	2x	–
858	2x	1.687	2.32	2x	–
858	2x	1.687	2.32	2x	–
858	2x	1.689	1.87	2x	–
858	2x	1.689	1.87	2x	–
858	2x	1.689	1.87	2x	–
858	2x	1.689	1.87	2x	–
858	2x	1.689	1.51	2x	–
858	2x	1.689	1.51	2x	–
858	2x	1.689	1.51	2x	–
858	2x	1.689	1.51	2x	–
858	2x	1.689	1.51	2x	–
858	2x	1.695	1.74	2x	–
858	2x	1.695	1.74	2x	–
858	2x	1.695	1.74	2x	–
858	2x	1.695	1.74	2x	–
858	2x	1.695	1.74	2x	–
858	2x	1.695	1.74	2x	–
858	2x	1.695	1.74	2x	–
858	2x	1.696	1.77	2x	–
858	2x	1.696	1.77	2x	–
858	2x	1.696	1.77	2x	–
858	2x	1.696	1.77	2x	–
858	2x	1.696	1.77	2x	–
858	2x	1.697	2.54	2x	–
858	2x	1.697	2.54	2x	–

858	2x	1.697	2.54	2x	–
858	2x	1.697	2.54	2x	–
858	2x	1.697	2.54	2x	–
858	2x	1.697	2.28	2x	–
858	2x	1.697	2.28	2x	–
858	2x	1.697	2.28	2x	–
858	2x	1.697	2.28	2x	–
858	2x	1.697	2.28	2x	–
858	2x	1.707	2.07	2x	–
858	2x	1.707	2.07	2x	–
858	2x	1.707	2.07	2x	–
858	2x	1.707	2.07	2x	–
858	2x	1.707	2.07	2x	–
858	2x	1.707	2.07	2x	–
858	2x	1.716	1.95	2x	–
858	2x	1.716	1.95	2x	–
858	2x	1.716	1.95	2x	–
858	2x	1.716	1.95	2x	–
858	2x	1.716	1.95	2x	–
858	2x	1.716	1.81	2x	–
858	2x	1.716	1.81	2x	–
858	2x	1.716	1.81	2x	–
858	2x	1.716	1.81	2x	–
858	2x	1.716	1.81	2x	–
299	4x	2.792	1.84	4x	–
299	4x	2.792	1.84	4x	–
299	4x	2.792	1.84	4x	–
299	4x	2.812	1.79	4x	–
299	4x	2.861	1.90	4x	–
299	4x	2.861	1.90	4x	–
299	4x	2.871	1.90	4x	–
299	4x	2.871	1.90	4x	–
299	4x	2.878	1.92	4x	–
299	4x	2.878	1.92	4x	–
299	4x	2.878	1.92	4x	–
299	4x	2.878	1.92	4x	–
299	4x	2.878	1.92	4x	–
299	4x	2.884	2.16	4x	–
299	4x	2.887	1.73	4x	–
299	4x	2.888	1.32	4x	–
299	4x	2.891	2.18	4x	–
299	4x	2.891	2.18	4x	–
299	4x	2.891	2.18	4x	–
299	4x	2.891	2.18	4x	–
299	4x	2.891	2.18	4x	–
299	4x	2.891	2.18	4x	–
299	4x	2.891	2.29	4x	–
299	4x	2.891	2.29	4x	–
299	4x	2.891	2.29	4x	–
299	4x	2.891	2.29	4x	–
299	4x	2.891	2.29	4x	–
299	4x	2.898	1.96	4x	–
299	4x	2.898	1.96	4x	–
299	4x	2.899	1.82	4x	–
299	4x	2.899	1.82	4x	–
299	4x	2.899	1.82	4x	–
299	4x	2.899	1.82	4x	–
299	4x	2.899	1.82	4x	–
299	4x	2.899	1.98	4x	–
299	4x	2.899	1.98	4x	–
299	4x	2.899	1.98	4x	–
299	4x	2.899	1.98	4x	–
299	4x	2.899	1.98	4x	–

299	4x	2.900	1.66	4x	–
299	4x	2.900	1.66	4x	–
299	4x	2.900	1.66	4x	–
299	4x	2.900	1.66	4x	–
299	4x	2.900	1.66	4x	–
299	4x	2.909	1.83	4x	–
299	4x	2.909	1.83	4x	–
299	4x	2.909	1.83	4x	–
299	4x	2.909	1.83	4x	–
299	4x	2.909	1.83	4x	–
299	4x	2.909	1.83	4x	–
299	4x	2.910	2.21	4x	–
299	4x	2.910	2.21	4x	–
299	4x	2.910	2.21	4x	–
299	4x	2.910	2.21	4x	–
299	4x	2.910	2.21	4x	–
299	4x	2.919	1.44	4x	–
299	4x	2.919	1.44	4x	–
299	4x	2.919	1.44	4x	–
299	4x	2.919	1.44	4x	–
299	4x	2.919	1.44	4x	–
299	4x	2.919	1.44	4x	–
299	4x	2.926	1.58	4x	–
299	4x	4.240	1.64	6x	2n = 6x = 54
22	2x + 3x + 4x	1.683	2.22	2x	–
22	2x + 3x + 4x	1.700	1.58	2x	–
22	2x + 3x + 4x	1.700	1.58	2x	–
22	2x + 3x + 4x	1.700	1.58	2x	–
22	2x + 3x + 4x	1.700	1.58	2x	–
22	2x + 3x + 4x	1.700	2.22	2x	–
22	2x + 3x + 4x	1.700	2.22	2x	–
22	2x + 3x + 4x	1.700	2.22	2x	–
22	2x + 3x + 4x	1.700	2.22	2x	–
22	2x + 3x + 4x	1.700	2.22	2x	–
22	2x + 3x + 4x	1.703	2.36	2x	–
22	2x + 3x + 4x	1.708	3.16	2x	–
22	2x + 3x + 4x	1.710	2.80	2x	–
22	2x + 3x + 4x	1.710	2.07	2x	–
22	2x + 3x + 4x	1.710	2.07	2x	–
22	2x + 3x + 4x	1.710	2.92	2x	–
22	2x + 3x + 4x	1.710	2.92	2x	–
22	2x + 3x + 4x	1.710	2.92	2x	–
22	2x + 3x + 4x	1.711	3.01	2x	–
22	2x + 3x + 4x	1.713	2.32	2x	2n = 2x = 18
22	2x + 3x + 4x	1.713	2.48	2x	–
22	2x + 3x + 4x	1.713	2.10	2x	–
22	2x + 3x + 4x	1.714	2.49	2x	–
22	2x + 3x + 4x	1.714	2.26	2x	–
22	2x + 3x + 4x	1.716	1.39	2x	–
22	2x + 3x + 4x	1.716	1.87	2x	–
22	2x + 3x + 4x	1.716	1.87	2x	–
22	2x + 3x + 4x	1.716	1.87	2x	–
22	2x + 3x + 4x	1.716	1.87	2x	–
22	2x + 3x + 4x	1.716	1.87	2x	–
22	2x + 3x + 4x	1.716	2.10	2x	–
22	2x + 3x + 4x	1.718	1.89	2x	–
22	2x + 3x + 4x	1.718	1.89	2x	–
22	2x + 3x + 4x	1.718	1.89	2x	–
22	2x + 3x + 4x	1.718	1.89	2x	–
22	2x + 3x + 4x	1.718	1.89	2x	–
22	2x + 3x + 4x	1.720	3.11	2x	–
22	2x + 3x + 4x	1.720	1.98	2x	–
22	2x + 3x + 4x	1.723	1.69	2x	–

22	2x + 3x + 4x	1.723	1.69	2x	–
22	2x + 3x + 4x	1.726	2.32	2x	–
22	2x + 3x + 4x	1.726	2.32	2x	–
22	2x + 3x + 4x	1.726	2.32	2x	–
22	2x + 3x + 4x	1.726	2.32	2x	–
22	2x + 3x + 4x	1.726	2.32	2x	–
22	2x + 3x + 4x	1.726	2.32	2x	–
22	2x + 3x + 4x	1.727	1.78	2x	–
22	2x + 3x + 4x	1.727	2.42	2x	–
22	2x + 3x + 4x	1.727	2.42	2x	–
22	2x + 3x + 4x	1.727	2.42	2x	–
22	2x + 3x + 4x	1.727	2.42	2x	–
22	2x + 3x + 4x	1.727	2.19	2x	–
22	2x + 3x + 4x	1.727	2.62	2x	–
22	2x + 3x + 4x	1.727	2.62	2x	–
22	2x + 3x + 4x	1.727	2.62	2x	–
22	2x + 3x + 4x	1.727	2.62	2x	–
22	2x + 3x + 4x	1.727	2.62	2x	2n = 2x = 18
22	2x + 3x + 4x	1.727	2.62	2x	–
22	2x + 3x + 4x	1.733	1.53	2x	–
22	2x + 3x + 4x	1.733	1.53	2x	–
22	2x + 3x + 4x	1.733	1.53	2x	–
22	2x + 3x + 4x	1.733	1.53	2x	–
22	2x + 3x + 4x	1.733	1.53	2x	–
22	2x + 3x + 4x	1.733	1.62	2x	–
22	2x + 3x + 4x	1.733	2.07	2x	–
22	2x + 3x + 4x	1.733	2.35	2x	–
22	2x + 3x + 4x	1.733	1.82	2x	–
22	2x + 3x + 4x	1.733	2.65	2x	–
22	2x + 3x + 4x	1.733	2.23	2x	–
22	2x + 3x + 4x	1.733	2.09	2x	–
22	2x + 3x + 4x	1.733	2.09	2x	–
22	2x + 3x + 4x	1.733	2.09	2x	–
22	2x + 3x + 4x	1.733	2.09	2x	–
22	2x + 3x + 4x	1.733	2.09	2x	–
22	2x + 3x + 4x	1.733	2.11	2x	–
22	2x + 3x + 4x	1.733	2.11	2x	–
22	2x + 3x + 4x	1.733	2.11	2x	–
22	2x + 3x + 4x	1.733	2.11	2x	–
22	2x + 3x + 4x	1.733	2.11	2x	–
22	2x + 3x + 4x	1.733	2.11	2x	–
22	2x + 3x + 4x	1.733	2.11	2x	–
22	2x + 3x + 4x	1.735	1.96	2x	–
22	2x + 3x + 4x	1.735	1.77	2x	–
22	2x + 3x + 4x	1.740	2.04	2x	–
22	2x + 3x + 4x	1.743	2.31	2x	–
22	2x + 3x + 4x	1.745	1.87	2x	–
22	2x + 3x + 4x	1.745	1.95	2x	–
22	2x + 3x + 4x	1.745	1.95	2x	–
22	2x + 3x + 4x	1.745	1.95	2x	–
22	2x + 3x + 4x	1.745	1.95	2x	–
22	2x + 3x + 4x	1.745	1.77	2x	–
22	2x + 3x + 4x	1.750	2.17	2x	–
22	2x + 3x + 4x	1.750	1.81	2x	–
22	2x + 3x + 4x	1.758	2.26	2x	–
22	2x + 3x + 4x	1.760	2.54	2x	–
22	2x + 3x + 4x	1.766	3.20	2x	–
22	2x + 3x + 4x	1.773	2.79	2x	–
22	2x + 3x + 4x	1.784	1.98	2x	–
22	2x + 3x + 4x	2.287	2.28	3x	–
22	2x + 3x + 4x	2.313	4.05	3x	–
22	2x + 3x + 4x	2.347	2.02	3x	–
22	2x + 3x + 4x	2.354	3.38	3x	–

22	2x + 3x + 4x	2.833	2.58	4x	–
22	2x + 3x + 4x	2.861	3.12	4x	–
22	2x + 3x + 4x	2.861	2.21	4x	–
22	2x + 3x + 4x	2.861	2.15	4x	–
22	2x + 3x + 4x	2.861	1.44	4x	–
22	2x + 3x + 4x	2.871	2.28	4x	–
22	2x + 3x + 4x	2.873	1.41	4x	–
22	2x + 3x + 4x	2.880	1.69	4x	–
22	2x + 3x + 4x	2.888	3.56	4x	2n = 4x = 36
22	2x + 3x + 4x	2.890	2.52	4x	–
22	2x + 3x + 4x	2.891	2.82	4x	–
22	2x + 3x + 4x	2.891	1.82	4x	–
22	2x + 3x + 4x	2.891	1.61	4x	–
22	2x + 3x + 4x	2.899	2.60	4x	–
22	2x + 3x + 4x	2.909	1.16	4x	–
22	2x + 3x + 4x	2.909	1.97	4x	–
22	2x + 3x + 4x	2.912	2.43	4x	–
22	2x + 3x + 4x	2.919	2.07	4x	–
22	2x + 3x + 4x	2.931	1.77	4x	–
22	2x + 3x + 4x	2.931	1.70	4x	–
22	2x + 3x + 4x	2.941	1.51	4x	–
22	2x + 3x + 4x	2.950	3.95	4x	–
22	2x + 3x + 4x	2.980	1.67	4x	–
22	2x + 3x + 4x	3.010	2.96	4x	–
46	2x + 3x + 4x	1.670	2.67	2x	–
46	2x + 3x + 4x	1.680	2.00	2x	–
46	2x + 3x + 4x	1.683	2.96	2x	–
46	2x + 3x + 4x	1.684	2.96	2x	–
46	2x + 3x + 4x	1.686	1.33	2x	–
46	2x + 3x + 4x	1.688	2.19	2x	–
46	2x + 3x + 4x	1.699	1.28	2x	–
46	2x + 3x + 4x	1.703	3.00	2x	–
46	2x + 3x + 4x	1.706	1.47	2x	–
46	2x + 3x + 4x	1.713	1.84	2x	–
46	2x + 3x + 4x	1.723	1.40	2x	–
46	2x + 3x + 4x	1.723	2.52	2x	–
46	2x + 3x + 4x	1.725	1.70	2x	–
46	2x + 3x + 4x	1.725	1.77	2x	–
46	2x + 3x + 4x	1.733	1.80	2x	–
46	2x + 3x + 4x	1.733	1.73	2x	–
46	2x + 3x + 4x	1.735	1.83	2x	–
46	2x + 3x + 4x	1.735	2.59	2x	–
46	2x + 3x + 4x	1.738	1.76	2x	–
46	2x + 3x + 4x	1.743	1.98	2x	–
46	2x + 3x + 4x	1.755	2.71	2x	–
46	2x + 3x + 4x	1.757	1.55	2x	–
46	2x + 3x + 4x	1.757	1.25	2x	–
46	2x + 3x + 4x	1.767	1.62	2x	–
46	2x + 3x + 4x	1.768	1.77	2x	–
46	2x + 3x + 4x	2.221	1.19	3x	–
46	2x + 3x + 4x	2.240	2.85	3x	–
46	2x + 3x + 4x	2.248	3.23	3x	–
46	2x + 3x + 4x	2.250	1.29	3x	–
46	2x + 3x + 4x	2.255	1.31	3x	–
46	2x + 3x + 4x	2.255	1.29	3x	2n = 3x = 27
46	2x + 3x + 4x	2.255	1.46	3x	–
46	2x + 3x + 4x	2.260	1.36	3x	–
46	2x + 3x + 4x	2.265	1.86	3x	–
46	2x + 3x + 4x	2.265	1.86	3x	–
46	2x + 3x + 4x	2.267	2.68	3x	–
46	2x + 3x + 4x	2.272	1.23	3x	–
46	2x + 3x + 4x	2.272	1.23	3x	–
46	2x + 3x + 4x	2.272	1.23	3x	–

46	2x + 3x + 4x	2.275	1.39	3x	–
46	2x + 3x + 4x	2.284	1.90	3x	–
46	2x + 3x + 4x	2.284	1.88	3x	–
46	2x + 3x + 4x	2.287	3.19	3x	–
46	2x + 3x + 4x	2.287	2.48	3x	–
46	2x + 3x + 4x	2.287	2.34	3x	–
46	2x + 3x + 4x	2.287	1.65	3x	2n = 3x = 27
46	2x + 3x + 4x	2.288	1.38	3x	–
46	2x + 3x + 4x	2.290	2.57	3x	–
46	2x + 3x + 4x	2.290	2.00	3x	–
46	2x + 3x + 4x	2.293	3.08	3x	–
46	2x + 3x + 4x	2.297	1.96	3x	–
46	2x + 3x + 4x	2.300	2.55	3x	–
46	2x + 3x + 4x	2.305	1.88	3x	–
46	2x + 3x + 4x	2.306	2.01	3x	–
46	2x + 3x + 4x	2.307	2.25	3x	–
46	2x + 3x + 4x	2.307	2.74	3x	–
46	2x + 3x + 4x	2.307	2.60	3x	–
46	2x + 3x + 4x	2.310	2.94	3x	–
46	2x + 3x + 4x	2.317	1.77	3x	–
46	2x + 3x + 4x	2.317	2.40	3x	–
46	2x + 3x + 4x	2.317	1.18	3x	–
46	2x + 3x + 4x	2.337	3.29	3x	–
46	2x + 3x + 4x	2.636	2.10	3x-4x	–
46	2x + 3x + 4x	2.780	2.07	4x	–
46	2x + 3x + 4x	2.796	1.64	4x	–
46	2x + 3x + 4x	2.800	1.61	4x	–
46	2x + 3x + 4x	2.812	1.23	4x	–
46	2x + 3x + 4x	2.814	1.20	4x	–
46	2x + 3x + 4x	2.824	1.54	4x	–
46	2x + 3x + 4x	2.825	1.83	4x	–
46	2x + 3x + 4x	2.825	1.83	4x	–
46	2x + 3x + 4x	2.825	1.83	4x	–
46	2x + 3x + 4x	2.825	1.83	4x	–
46	2x + 3x + 4x	2.825	1.83	4x	–
46	2x + 3x + 4x	2.832	2.71	4x	–
46	2x + 3x + 4x	2.832	1.44	4x	–
46	2x + 3x + 4x	2.833	1.91	4x	–
46	2x + 3x + 4x	2.833	1.62	4x	–
46	2x + 3x + 4x	2.833	1.29	4x	–
46	2x + 3x + 4x	2.835	1.13	4x	–
46	2x + 3x + 4x	2.835	1.34	4x	–
46	2x + 3x + 4x	2.835	1.34	4x	–
46	2x + 3x + 4x	2.835	1.34	4x	–
46	2x + 3x + 4x	2.840	2.11	4x	–
46	2x + 3x + 4x	2.841	1.34	4x	–
46	2x + 3x + 4x	2.842	1.57	4x	–
46	2x + 3x + 4x	2.842	1.52	4x	–
46	2x + 3x + 4x	2.843	1.37	4x	–
46	2x + 3x + 4x	2.849	1.47	4x	–
46	2x + 3x + 4x	2.850	1.99	4x	–
46	2x + 3x + 4x	2.851	2.18	4x	–
46	2x + 3x + 4x	2.851	1.44	4x	–
46	2x + 3x + 4x	2.853	1.79	4x	–
46	2x + 3x + 4x	2.853	1.43	4x	–
46	2x + 3x + 4x	2.853	1.43	4x	–
46	2x + 3x + 4x	2.853	1.43	4x	2n = 4x = 36
46	2x + 3x + 4x	2.854	1.21	4x	–
46	2x + 3x + 4x	2.854	1.22	4x	–
46	2x + 3x + 4x	2.854	1.26	4x	–
46	2x + 3x + 4x	2.859	1.96	4x	–
46	2x + 3x + 4x	2.859	1.96	4x	–
46	2x + 3x + 4x	2.859	1.96	4x	–

46	2x + 3x + 4x	2.859	1.96	4x	–
46	2x + 3x + 4x	2.859	1.96	4x	–
46	2x + 3x + 4x	2.859	1.96	4x	–
46	2x + 3x + 4x	2.860	2.53	4x	–
46	2x + 3x + 4x	2.861	2.08	4x	–
46	2x + 3x + 4x	2.861	1.62	4x	–
46	2x + 3x + 4x	2.861	1.97	4x	–
46	2x + 3x + 4x	2.861	1.98	4x	–
46	2x + 3x + 4x	2.861	1.98	4x	–
46	2x + 3x + 4x	2.861	1.98	4x	–
46	2x + 3x + 4x	2.861	1.98	4x	–
46	2x + 3x + 4x	2.861	1.98	4x	–
46	2x + 3x + 4x	2.861	1.97	4x	–
46	2x + 3x + 4x	2.863	1.66	4x	–
46	2x + 3x + 4x	2.863	1.26	4x	–
46	2x + 3x + 4x	2.863	1.28	4x	–
46	2x + 3x + 4x	2.863	1.28	4x	–
46	2x + 3x + 4x	2.863	1.20	4x	–
46	2x + 3x + 4x	2.864	1.21	4x	–
46	2x + 3x + 4x	2.865	1.27	4x	–
46	2x + 3x + 4x	2.870	1.79	4x	–
46	2x + 3x + 4x	2.871	1.62	4x	–
46	2x + 3x + 4x	2.871	1.74	4x	–
46	2x + 3x + 4x	2.871	1.77	4x	–
46	2x + 3x + 4x	2.871	1.52	4x	–
46	2x + 3x + 4x	2.871	1.52	4x	–
46	2x + 3x + 4x	2.871	1.50	4x	–
46	2x + 3x + 4x	2.871	1.50	4x	–
46	2x + 3x + 4x	2.873	1.59	4x	–
46	2x + 3x + 4x	2.873	1.38	4x	–
46	2x + 3x + 4x	2.873	1.71	4x	–
46	2x + 3x + 4x	2.873	1.71	4x	–
46	2x + 3x + 4x	2.873	1.71	4x	–
46	2x + 3x + 4x	2.873	1.71	4x	–
46	2x + 3x + 4x	2.875	1.42	4x	–
46	2x + 3x + 4x	2.876	1.90	4x	–
46	2x + 3x + 4x	2.877	1.17	4x	–
46	2x + 3x + 4x	2.877	2.42	4x	–
46	2x + 3x + 4x	2.878	1.78	4x	–
46	2x + 3x + 4x	2.879	2.73	4x	–
46	2x + 3x + 4x	2.881	1.74	4x	–
46	2x + 3x + 4x	2.881	2.58	4x	–
46	2x + 3x + 4x	2.881	1.64	4x	–
46	2x + 3x + 4x	2.881	1.91	4x	–
46	2x + 3x + 4x	2.881	1.91	4x	–
46	2x + 3x + 4x	2.881	1.91	4x	–
46	2x + 3x + 4x	2.881	1.91	4x	–
46	2x + 3x + 4x	2.881	1.91	4x	–
46	2x + 3x + 4x	2.881	1.91	4x	–
46	2x + 3x + 4x	2.882	1.60	4x	–
46	2x + 3x + 4x	2.883	1.79	4x	–
46	2x + 3x + 4x	2.883	1.79	4x	–
46	2x + 3x + 4x	2.883	1.79	4x	–
46	2x + 3x + 4x	2.883	1.19	4x	–
46	2x + 3x + 4x	2.887	2.87	4x	2n = 4x = 36
46	2x + 3x + 4x	2.888	2.01	4x	–
46	2x + 3x + 4x	2.889	1.71	4x	–
46	2x + 3x + 4x	2.889	1.92	4x	–
46	2x + 3x + 4x	2.889	2.14	4x	–
46	2x + 3x + 4x	2.890	1.78	4x	–
46	2x + 3x + 4x	2.890	1.78	4x	–
46	2x + 3x + 4x	2.890	1.78	4x	–
46	2x + 3x + 4x	2.890	1.78	4x	–

46	2x + 3x + 4x	2.890	1.78	4x	–
46	2x + 3x + 4x	2.890	1.78	4x	–
46	2x + 3x + 4x	2.890	1.78	4x	–
46	2x + 3x + 4x	2.890	2.54	4x	–
46	2x + 3x + 4x	2.890	1.55	4x	–
46	2x + 3x + 4x	2.890	1.55	4x	–
46	2x + 3x + 4x	2.890	1.55	4x	–
46	2x + 3x + 4x	2.890	1.55	4x	–
46	2x + 3x + 4x	2.890	1.55	4x	–
46	2x + 3x + 4x	2.890	1.55	4x	–
46	2x + 3x + 4x	2.890	1.55	4x	–
46	2x + 3x + 4x	2.891	2.03	4x	–
46	2x + 3x + 4x	2.891	1.65	4x	–
46	2x + 3x + 4x	2.891	1.67	4x	–
46	2x + 3x + 4x	2.891	2.15	4x	–
46	2x + 3x + 4x	2.891	2.15	4x	–
46	2x + 3x + 4x	2.891	2.15	4x	–
46	2x + 3x + 4x	2.891	2.15	4x	–
46	2x + 3x + 4x	2.891	2.15	4x	–
46	2x + 3x + 4x	2.891	1.59	4x	–
46	2x + 3x + 4x	2.891	1.45	4x	–
46	2x + 3x + 4x	2.891	1.45	4x	–
46	2x + 3x + 4x	2.891	1.45	4x	–
46	2x + 3x + 4x	2.891	1.45	4x	–
46	2x + 3x + 4x	2.891	1.45	4x	–
46	2x + 3x + 4x	2.891	1.45	4x	–
46	2x + 3x + 4x	2.892	1.70	4x	–
46	2x + 3x + 4x	2.892	1.67	4x	–
46	2x + 3x + 4x	2.892	1.74	4x	–
46	2x + 3x + 4x	2.892	1.39	4x	–
46	2x + 3x + 4x	2.892	1.73	4x	–
46	2x + 3x + 4x	2.892	1.18	4x	–
46	2x + 3x + 4x	2.894	1.56	4x	–
46	2x + 3x + 4x	2.899	2.48	4x	–
46	2x + 3x + 4x	2.900	2.12	4x	–
46	2x + 3x + 4x	2.900	2.15	4x	–
46	2x + 3x + 4x	2.900	2.15	4x	2n = 4x = 36
46	2x + 3x + 4x	2.900	2.15	4x	–
46	2x + 3x + 4x	2.900	2.15	4x	–
46	2x + 3x + 4x	2.900	2.15	4x	–
46	2x + 3x + 4x	2.900	2.13	4x	–
46	2x + 3x + 4x	2.901	3.07	4x	–
46	2x + 3x + 4x	2.901	1.62	4x	–
46	2x + 3x + 4x	2.901	1.68	4x	–
46	2x + 3x + 4x	2.901	1.68	4x	–
46	2x + 3x + 4x	2.901	1.68	4x	–
46	2x + 3x + 4x	2.901	1.68	4x	–
46	2x + 3x + 4x	2.901	1.68	4x	–
46	2x + 3x + 4x	2.901	2.32	4x	–
46	2x + 3x + 4x	2.901	2.32	4x	–
46	2x + 3x + 4x	2.901	2.32	4x	–
46	2x + 3x + 4x	2.901	2.32	4x	–
46	2x + 3x + 4x	2.901	2.32	4x	–
46	2x + 3x + 4x	2.902	2.23	4x	–
46	2x + 3x + 4x	2.902	2.23	4x	–
46	2x + 3x + 4x	2.902	2.23	4x	–
46	2x + 3x + 4x	2.902	2.23	4x	–
46	2x + 3x + 4x	2.902	1.29	4x	–
46	2x + 3x + 4x	2.902	1.29	4x	–
46	2x + 3x + 4x	2.902	1.29	4x	–
46	2x + 3x + 4x	2.902	1.29	4x	–
46	2x + 3x + 4x	2.905	2.04	4x	–
46	2x + 3x + 4x	2.908	1.91	4x	–

46	2x + 3x + 4x	2.910	2.16	4x	–
46	2x + 3x + 4x	2.911	2.31	4x	–
46	2x + 3x + 4x	2.913	1.44	4x	–
46	2x + 3x + 4x	2.913	2.57	4x	–
46	2x + 3x + 4x	2.918	1.61	4x	–
46	2x + 3x + 4x	2.919	2.84	4x	–
46	2x + 3x + 4x	2.919	2.84	4x	–
46	2x + 3x + 4x	2.919	2.84	4x	–
46	2x + 3x + 4x	2.920	2.08	4x	–
46	2x + 3x + 4x	2.920	2.89	4x	–
46	2x + 3x + 4x	2.920	2.77	4x	–
46	2x + 3x + 4x	2.920	2.77	4x	–
46	2x + 3x + 4x	2.920	2.77	4x	–
46	2x + 3x + 4x	2.922	2.94	4x	–
46	2x + 3x + 4x	2.931	2.30	4x	–
46	2x + 3x + 4x	2.931	1.58	4x	–
46	2x + 3x + 4x	2.931	2.66	4x	–
46	2x + 3x + 4x	2.941	1.76	4x	–
46	2x + 3x + 4x	2.941	2.10	4x	–
46	2x + 3x + 4x	2.950	1.59	4x	–
46	2x + 3x + 4x	2.950	1.39	4x	–
46	2x + 3x + 4x	2.961	1.13	4x	–
46	2x + 3x + 4x	2.979	3.10	4x	–
46	2x + 3x + 4x	2.979	3.10	4x	–
46	2x + 3x + 4x	2.979	3.10	4x	–
46	2x + 3x + 4x	2.979	3.10	4x	–
46	2x + 3x + 4x	2.979	3.10	4x	–
46	2x + 3x + 4x	2.980	1.89	4x	–
46	2x + 3x + 4x	2.980	1.76	4x	–
46	2x + 3x + 4x	2.990	1.99	4x	–
46	2x + 3x + 4x	3.020	1.89	4x	–
46	2x + 3x + 4x	3.040	1.68	4x	–
66	2x + 3x + 4x	1.693	1.43	2x	–
66	2x + 3x + 4x	1.698	1.52	2x	–
66	2x + 3x + 4x	1.698	1.63	2x	–
66	2x + 3x + 4x	1.702	1.34	2x	–
66	2x + 3x + 4x	1.702	1.34	2x	–
66	2x + 3x + 4x	1.703	1.70	2x	–
66	2x + 3x + 4x	1.703	2.34	2x	–
66	2x + 3x + 4x	1.706	1.43	2x	–
66	2x + 3x + 4x	1.714	1.73	2x	–
66	2x + 3x + 4x	1.723	1.88	2x	–
66	2x + 3x + 4x	1.723	2.18	2x	–
66	2x + 3x + 4x	1.723	2.79	2x	–
66	2x + 3x + 4x	1.724	3.77	2x	–
66	2x + 3x + 4x	1.725	2.23	2x	–
66	2x + 3x + 4x	1.725	2.23	2x	–
66	2x + 3x + 4x	1.728	1.61	2x	–
66	2x + 3x + 4x	1.730	1.54	2x	–
66	2x + 3x + 4x	1.730	3.92	2x	–
66	2x + 3x + 4x	1.733	1.70	2x	–
66	2x + 3x + 4x	1.733	1.63	2x	–
66	2x + 3x + 4x	1.733	1.63	2x	–
66	2x + 3x + 4x	1.733	1.67	2x	–
66	2x + 3x + 4x	1.733	1.67	2x	–
66	2x + 3x + 4x	1.733	1.67	2x	–
66	2x + 3x + 4x	1.733	1.67	2x	–
66	2x + 3x + 4x	1.735	1.80	2x	–
66	2x + 3x + 4x	1.735	1.63	2x	–
66	2x + 3x + 4x	1.735	1.28	2x	–
66	2x + 3x + 4x	1.735	1.28	2x	–
66	2x + 3x + 4x	1.737	1.71	2x	–
66	2x + 3x + 4x	1.737	1.71	2x	–

66	2x + 3x + 4x	1.740	3.82	2x	–
66	2x + 3x + 4x	1.740	2.15	2x	–
66	2x + 3x + 4x	1.740	2.15	2x	–
66	2x + 3x + 4x	1.740	2.12	2x	–
66	2x + 3x + 4x	1.740	1.75	2x	–
66	2x + 3x + 4x	1.740	1.75	2x	–
66	2x + 3x + 4x	1.740	1.75	2x	–
66	2x + 3x + 4x	1.743	1.87	2x	–
66	2x + 3x + 4x	1.743	1.87	2x	–
66	2x + 3x + 4x	1.743	1.76	2x	–
66	2x + 3x + 4x	1.743	1.40	2x	–
66	2x + 3x + 4x	1.743	1.40	2x	–
66	2x + 3x + 4x	1.743	2.08	2x	–
66	2x + 3x + 4x	1.743	2.08	2x	–
66	2x + 3x + 4x	1.743	1.94	2x	–
66	2x + 3x + 4x	1.743	1.94	2x	–
66	2x + 3x + 4x	1.745	1.85	2x	–
66	2x + 3x + 4x	1.745	1.85	2x	–
66	2x + 3x + 4x	1.745	1.85	2x	–
66	2x + 3x + 4x	1.755	1.85	2x	–
66	2x + 3x + 4x	1.755	2.12	2x	–
66	2x + 3x + 4x	1.755	1.96	2x	–
66	2x + 3x + 4x	1.755	2.89	2x	–
66	2x + 3x + 4x	1.755	2.89	2x	–
66	2x + 3x + 4x	1.765	1.81	2x	–
66	2x + 3x + 4x	1.770	2.82	2x	–
66	2x + 3x + 4x	2.291	1.42	3x	–
66	2x + 3x + 4x	2.297	4.13	3x	–
66	2x + 3x + 4x	2.297	3.49	3x	–
66	2x + 3x + 4x	2.306	3.39	3x	–
66	2x + 3x + 4x	2.314	1.76	3x	–
66	2x + 3x + 4x	2.317	2.87	3x	–
66	2x + 3x + 4x	2.320	3.54	3x	–
66	2x + 3x + 4x	2.354	3.60	3x	–
66	2x + 3x + 4x	2.366	3.57	3x	–
66	2x + 3x + 4x	2.860	1.73	4x	–
66	2x + 3x + 4x	2.861	2.35	4x	–
66	2x + 3x + 4x	2.861	2.35	4x	–
66	2x + 3x + 4x	2.861	2.35	4x	–
66	2x + 3x + 4x	2.861	2.35	4x	–
66	2x + 3x + 4x	2.861	1.42	4x	–
66	2x + 3x + 4x	2.869	3.22	4x	–
66	2x + 3x + 4x	2.871	2.85	4x	–
66	2x + 3x + 4x	2.871	2.85	4x	–
66	2x + 3x + 4x	2.871	2.85	4x	–
66	2x + 3x + 4x	2.871	2.85	4x	–
66	2x + 3x + 4x	2.871	2.85	4x	–
66	2x + 3x + 4x	2.881	1.53	4x	–
66	2x + 3x + 4x	2.881	1.53	4x	–
66	2x + 3x + 4x	2.881	1.53	4x	–
66	2x + 3x + 4x	2.881	1.53	4x	–
66	2x + 3x + 4x	2.881	1.53	4x	–
66	2x + 3x + 4x	2.881	1.84	4x	–
66	2x + 3x + 4x	2.881	1.84	4x	–
66	2x + 3x + 4x	2.881	1.84	4x	–
66	2x + 3x + 4x	2.885	1.44	4x	–
66	2x + 3x + 4x	2.891	3.09	4x	–
66	2x + 3x + 4x	2.891	1.75	4x	–
66	2x + 3x + 4x	2.891	1.75	4x	–
66	2x + 3x + 4x	2.891	1.75	4x	–
66	2x + 3x + 4x	2.892	1.85	4x	–
66	2x + 3x + 4x	2.892	1.85	4x	–
66	2x + 3x + 4x	2.892	1.85	4x	–

66	2x + 3x + 4x	2.910	2.32	4x	–
66	2x + 3x + 4x	2.910	2.32	4x	–
66	2x + 3x + 4x	2.910	2.32	4x	–
66	2x + 3x + 4x	2.910	2.32	4x	–
66	2x + 3x + 4x	2.911	2.40	4x	–
66	2x + 3x + 4x	2.911	2.40	4x	–
66	2x + 3x + 4x	2.911	2.40	4x	–
66	2x + 3x + 4x	2.911	2.40	4x	–
66	2x + 3x + 4x	2.911	2.40	4x	–
66	2x + 3x + 4x	2.911	1.99	4x	–
66	2x + 3x + 4x	2.911	1.99	4x	–
66	2x + 3x + 4x	2.911	1.99	4x	–
66	2x + 3x + 4x	2.911	1.99	4x	–
66	2x + 3x + 4x	2.911	1.99	4x	–
66	2x + 3x + 4x	2.912	1.67	4x	–
66	2x + 3x + 4x	2.912	1.51	4x	–
66	2x + 3x + 4x	2.912	1.51	4x	–
66	2x + 3x + 4x	2.912	1.51	4x	–
66	2x + 3x + 4x	2.919	3.70	4x	–
66	2x + 3x + 4x	2.919	3.02	4x	–
66	2x + 3x + 4x	2.920	1.32	4x	–
66	2x + 3x + 4x	2.921	1.45	4x	–
66	2x + 3x + 4x	2.921	1.45	4x	–
66	2x + 3x + 4x	2.921	1.45	4x	–
66	2x + 3x + 4x	2.921	1.16	4x	–
66	2x + 3x + 4x	2.922	1.27	4x	–
66	2x + 3x + 4x	2.922	1.27	4x	–
66	2x + 3x + 4x	2.922	1.27	4x	–
66	2x + 3x + 4x	2.922	1.33	4x	–
66	2x + 3x + 4x	2.922	1.33	4x	–
66	2x + 3x + 4x	2.922	1.37	4x	–
66	2x + 3x + 4x	2.922	1.49	4x	–
66	2x + 3x + 4x	2.922	1.49	4x	–
66	2x + 3x + 4x	2.922	1.49	4x	–
66	2x + 3x + 4x	2.922	1.49	4x	–
66	2x + 3x + 4x	2.929	3.12	4x	–
66	2x + 3x + 4x	2.931	1.82	4x	–
66	2x + 3x + 4x	2.931	1.82	4x	–
66	2x + 3x + 4x	2.931	1.82	4x	–
66	2x + 3x + 4x	2.931	1.47	4x	–
66	2x + 3x + 4x	2.931	1.47	4x	–
66	2x + 3x + 4x	2.931	2.40	4x	–
66	2x + 3x + 4x	2.931	1.84	4x	–
66	2x + 3x + 4x	2.931	1.84	4x	–
66	2x + 3x + 4x	2.931	1.84	4x	–
66	2x + 3x + 4x	2.931	1.70	4x	–
66	2x + 3x + 4x	2.931	1.70	4x	–
66	2x + 3x + 4x	2.931	1.70	4x	–
66	2x + 3x + 4x	2.931	1.55	4x	–
66	2x + 3x + 4x	2.931	1.55	4x	–
66	2x + 3x + 4x	2.939	3.09	4x	–
66	2x + 3x + 4x	2.939	2.47	4x	–
66	2x + 3x + 4x	2.941	1.54	4x	–
66	2x + 3x + 4x	2.949	2.91	4x	–
66	2x + 3x + 4x	2.949	1.68	4x	–
66	2x + 3x + 4x	2.949	1.68	4x	–
66	2x + 3x + 4x	2.949	1.68	4x	–
66	2x + 3x + 4x	2.958	2.15	4x	–
66	2x + 3x + 4x	2.958	2.15	4x	–
66	2x + 3x + 4x	2.958	2.15	4x	–
66	2x + 3x + 4x	2.959	3.00	4x	–
66	2x + 3x + 4x	2.960	3.40	4x	2n = 4x = 36
66	2x + 3x + 4x	2.960	2.73	4x	–

66	2x + 3x + 4x	2.960	2.10	4x	–
66	2x + 3x + 4x	2.960	2.58	4x	–
66	2x + 3x + 4x	2.969	4.05	4x	–
66	2x + 3x + 4x	2.969	1.81	4x	–
66	2x + 3x + 4x	2.969	1.81	4x	–
66	2x + 3x + 4x	2.969	2.15	4x	–
66	2x + 3x + 4x	2.969	2.15	4x	–
66	2x + 3x + 4x	2.970	2.38	4x	–
66	2x + 3x + 4x	2.970	2.49	4x	–
66	2x + 3x + 4x	2.970	2.49	4x	–
66	2x + 3x + 4x	2.970	2.49	4x	–
66	2x + 3x + 4x	2.970	2.49	4x	–
66	2x + 3x + 4x	2.971	1.59	4x	–
66	2x + 3x + 4x	2.980	2.17	4x	–
66	2x + 3x + 4x	2.980	2.17	4x	–
66	2x + 3x + 4x	2.980	2.17	4x	–
66	2x + 3x + 4x	3.000	3.04	4x	–
66	2x + 3x + 4x	3.000	2.32	4x	–
66	2x + 3x + 4x	3.020	1.94	4x	–
220	2x + 3x + 4x	1.699	1.48	2x	–
220	2x + 3x + 4x	1.716	1.35	2x	–
220	2x + 3x + 4x	1.730	2.02	2x	–
220	2x + 3x + 4x	1.733	1.89	2x	–
220	2x + 3x + 4x	1.733	1.89	2x	–
220	2x + 3x + 4x	1.733	1.89	2x	–
220	2x + 3x + 4x	1.738	1.34	2x	–
220	2x + 3x + 4x	1.970	1.37	2x-3x	–
220	2x + 3x + 4x	2.245	2.26	3x	–
220	2x + 3x + 4x	2.245	1.62	3x	–
220	2x + 3x + 4x	2.248	1.61	3x	–
220	2x + 3x + 4x	2.255	1.39	3x	–
220	2x + 3x + 4x	2.257	1.53	3x	–
220	2x + 3x + 4x	2.262	3.72	3x	–
220	2x + 3x + 4x	2.267	1.19	3x	–
220	2x + 3x + 4x	2.267	1.39	3x	–
220	2x + 3x + 4x	2.272	1.27	3x	–
220	2x + 3x + 4x	2.272	1.45	3x	–
220	2x + 3x + 4x	2.273	1.36	3x	–
220	2x + 3x + 4x	2.275	1.74	3x	–
220	2x + 3x + 4x	2.275	1.44	3x	–
220	2x + 3x + 4x	2.277	1.70	3x	–
220	2x + 3x + 4x	2.277	1.34	3x	–
220	2x + 3x + 4x	2.282	2.28	3x	–
220	2x + 3x + 4x	2.283	2.45	3x	–
220	2x + 3x + 4x	2.283	4.26	3x	–
220	2x + 3x + 4x	2.283	3.04	3x	–
220	2x + 3x + 4x	2.283	1.75	3x	–
220	2x + 3x + 4x	2.283	1.75	3x	–
220	2x + 3x + 4x	2.283	1.75	3x	–
220	2x + 3x + 4x	2.283	1.75	3x	–
220	2x + 3x + 4x	2.283	1.75	3x	–
220	2x + 3x + 4x	2.284	1.35	3x	–
220	2x + 3x + 4x	2.287	1.33	3x	–
220	2x + 3x + 4x	2.291	3.30	3x	–
220	2x + 3x + 4x	2.292	2.40	3x	–
220	2x + 3x + 4x	2.293	3.10	3x	–
220	2x + 3x + 4x	2.294	3.34	3x	–
220	2x + 3x + 4x	2.304	1.34	3x	–
220	2x + 3x + 4x	2.313	1.73	3x	–
220	2x + 3x + 4x	2.314	1.32	3x	–
220	2x + 3x + 4x	2.317	1.83	3x	–
220	2x + 3x + 4x	2.427	2.23	3x-4x	–
220	2x + 3x + 4x	2.436	1.78	3x-4x	2n = 3x+2 = 29

220	2x + 3x + 4x	2.446	1.36	3x-4x	–
220	2x + 3x + 4x	2.495	1.97	3x-4x	–
220	2x + 3x + 4x	2.525	1.54	3x-4x	–
220	2x + 3x + 4x	2.539	1.30	3x-4x	–
220	2x + 3x + 4x	2.574	1.56	3x-4x	–
220	2x + 3x + 4x	2.612	1.49	3x-4x	–
220	2x + 3x + 4x	2.622	2.53	3x-4x	–
220	2x + 3x + 4x	2.624	2.40	3x-4x	–
220	2x + 3x + 4x	2.640	2.35	3x-4x	–
220	2x + 3x + 4x	2.663	2.05	3x-4x	–
220	2x + 3x + 4x	2.663	2.05	3x-4x	–
220	2x + 3x + 4x	2.663	2.05	3x-4x	–
220	2x + 3x + 4x	2.663	1.33	3x-4x	–
220	2x + 3x + 4x	2.683	1.31	3x-4x	–
220	2x + 3x + 4x	2.717	2.77	3x-4x	–
220	2x + 3x + 4x	2.719	1.97	3x-4x	–
220	2x + 3x + 4x	2.723	2.54	3x-4x	–
220	2x + 3x + 4x	2.723	2.54	3x-4x	–
220	2x + 3x + 4x	2.723	2.54	3x-4x	–
220	2x + 3x + 4x	2.723	2.54	3x-4x	–
220	2x + 3x + 4x	2.733	1.21	3x-4x	–
220	2x + 3x + 4x	2.735	2.57	3x-4x	–
220	2x + 3x + 4x	2.735	2.04	3x-4x	–
220	2x + 3x + 4x	2.760	1.82	3x-4x	–
220	2x + 3x + 4x	2.772	1.27	4x	–
220	2x + 3x + 4x	2.804	1.94	4x	–
220	2x + 3x + 4x	2.820	2.83	4x	–
220	2x + 3x + 4x	2.843	1.64	4x	–
220	2x + 3x + 4x	2.863	1.48	4x	–
220	2x + 3x + 4x	2.871	1.77	4x	–
220	2x + 3x + 4x	2.881	2.06	4x	–
220	2x + 3x + 4x	2.883	1.30	4x	–
220	2x + 3x + 4x	2.898	2.78	4x	–
220	2x + 3x + 4x	2.900	2.12	4x	–
220	2x + 3x + 4x	2.920	2.83	4x	–
220	2x + 3x + 4x	2.931	1.94	4x	–
220	2x + 3x + 4x	3.262	1.18	4x-5x	2n = 4x+5 = 41
220	2x + 3x + 4x	3.624	1.11	5x	–
323	2x + 3x + 4x	1.723	3.27	2x	–
323	2x + 3x + 4x	1.733	2.53	2x	–
323	2x + 3x + 4x	1.743	2.00	2x	–
323	2x + 3x + 4x	1.743	2.74	2x	–
323	2x + 3x + 4x	1.743	1.73	2x	–
323	2x + 3x + 4x	1.743	1.73	2x	–
323	2x + 3x + 4x	1.745	2.74	2x	–
323	2x + 3x + 4x	1.745	1.65	2x	–
323	2x + 3x + 4x	1.745	1.65	2x	–
323	2x + 3x + 4x	1.747	3.62	2x	–
323	2x + 3x + 4x	1.750	2.49	2x	–
323	2x + 3x + 4x	1.750	3.12	2x	–
323	2x + 3x + 4x	1.752	2.11	2x	–
323	2x + 3x + 4x	1.752	1.57	2x	–
323	2x + 3x + 4x	1.752	1.57	2x	–
323	2x + 3x + 4x	1.765	1.32	2x	–
323	2x + 3x + 4x	1.768	2.22	2x	–
323	2x + 3x + 4x	1.794	2.58	2x	–
323	2x + 3x + 4x	2.267	1.18	3x	–
323	2x + 3x + 4x	2.275	2.11	3x	–
323	2x + 3x + 4x	2.287	2.24	3x	–
323	2x + 3x + 4x	2.294	1.34	3x	2n = 3x = 27
323	2x + 3x + 4x	2.313	3.29	3x	–
323	2x + 3x + 4x	2.317	1.34	3x	–
323	2x + 3x + 4x	2.327	2.10	3x	–

323	2x + 3x + 4x	2.384	1.86	3x-4x	–
323	2x + 3x + 4x	2.396	3.23	3x-4x	–
323	2x + 3x + 4x	2.398	3.16	3x-4x	–
323	2x + 3x + 4x	2.673	2.69	3x-4x	–
323	2x + 3x + 4x	2.790	2.99	4x	–
323	2x + 3x + 4x	2.806	1.90	4x	–
323	2x + 3x + 4x	2.825	1.63	4x	–
323	2x + 3x + 4x	2.825	1.81	4x	–
323	2x + 3x + 4x	2.843	2.57	4x	–
323	2x + 3x + 4x	2.850	1.36	4x	–
323	2x + 3x + 4x	2.851	1.48	4x	–
323	2x + 3x + 4x	2.860	3.22	4x	–
323	2x + 3x + 4x	2.861	1.74	4x	–
323	2x + 3x + 4x	2.861	1.97	4x	–
323	2x + 3x + 4x	2.863	1.22	4x	–
323	2x + 3x + 4x	2.867	3.43	4x	–
323	2x + 3x + 4x	2.868	1.55	4x	–
323	2x + 3x + 4x	2.868	1.55	4x	–
323	2x + 3x + 4x	2.868	1.55	4x	–
323	2x + 3x + 4x	2.868	1.55	4x	–
323	2x + 3x + 4x	2.868	1.55	4x	–
323	2x + 3x + 4x	2.870	1.85	4x	–
323	2x + 3x + 4x	2.871	2.34	4x	–
323	2x + 3x + 4x	2.871	1.64	4x	–
323	2x + 3x + 4x	2.871	1.64	4x	–
323	2x + 3x + 4x	2.871	1.64	4x	–
323	2x + 3x + 4x	2.871	1.64	4x	–
323	2x + 3x + 4x	2.871	1.64	4x	–
323	2x + 3x + 4x	2.874	1.07	4x	–
323	2x + 3x + 4x	2.876	2.02	4x	–
323	2x + 3x + 4x	2.881	2.99	4x	–
323	2x + 3x + 4x	2.881	1.25	4x	–
323	2x + 3x + 4x	2.883	1.11	4x	–
323	2x + 3x + 4x	2.885	1.66	4x	–
323	2x + 3x + 4x	2.885	1.66	4x	–
323	2x + 3x + 4x	2.885	1.66	4x	–
323	2x + 3x + 4x	2.885	1.66	4x	–
323	2x + 3x + 4x	2.885	1.66	4x	–
323	2x + 3x + 4x	2.885	1.66	4x	–
323	2x + 3x + 4x	2.885	1.87	4x	–
323	2x + 3x + 4x	2.887	2.51	4x	–
323	2x + 3x + 4x	2.887	3.64	4x	–
323	2x + 3x + 4x	2.890	1.59	4x	–
323	2x + 3x + 4x	2.890	2.99	4x	–
323	2x + 3x + 4x	2.890	2.99	4x	–
323	2x + 3x + 4x	2.890	2.15	4x	–
323	2x + 3x + 4x	2.890	2.15	4x	–
323	2x + 3x + 4x	2.890	2.15	4x	–
323	2x + 3x + 4x	2.890	2.15	4x	–
323	2x + 3x + 4x	2.890	2.99	4x	–
323	2x + 3x + 4x	2.890	2.99	4x	–
323	2x + 3x + 4x	2.891	1.86	4x	–
323	2x + 3x + 4x	2.891	2.15	4x	–
323	2x + 3x + 4x	2.891	2.15	4x	–
323	2x + 3x + 4x	2.891	2.15	4x	–
323	2x + 3x + 4x	2.891	2.15	4x	–
323	2x + 3x + 4x	2.891	1.56	4x	–
323	2x + 3x + 4x	2.893	1.19	4x	–
323	2x + 3x + 4x	2.894	2.03	4x	–
323	2x + 3x + 4x	2.894	2.03	4x	–
323	2x + 3x + 4x	2.894	2.03	4x	–
323	2x + 3x + 4x	2.894	2.03	4x	–
323	2x + 3x + 4x	2.894	2.03	4x	–

323	2x + 3x + 4x	2.894	1.96	4x	–
323	2x + 3x + 4x	2.894	1.96	4x	–
323	2x + 3x + 4x	2.894	1.96	4x	–
323	2x + 3x + 4x	2.894	1.96	4x	–
323	2x + 3x + 4x	2.894	1.16	4x	–
323	2x + 3x + 4x	2.899	1.92	4x	–
323	2x + 3x + 4x	2.899	2.45	4x	–
323	2x + 3x + 4x	2.899	1.14	4x	–
323	2x + 3x + 4x	2.900	1.90	4x	–
323	2x + 3x + 4x	2.901	2.34	4x	–
323	2x + 3x + 4x	2.901	2.34	4x	–
323	2x + 3x + 4x	2.901	2.34	4x	–
323	2x + 3x + 4x	2.901	2.34	4x	–
323	2x + 3x + 4x	2.901	2.34	4x	–
323	2x + 3x + 4x	2.901	2.34	4x	–
323	2x + 3x + 4x	2.902	1.75	4x	–
323	2x + 3x + 4x	2.902	1.75	4x	–
323	2x + 3x + 4x	2.902	1.75	4x	–
323	2x + 3x + 4x	2.902	1.75	4x	–
323	2x + 3x + 4x	2.902	1.16	4x	–
323	2x + 3x + 4x	2.903	2.04	4x	–
323	2x + 3x + 4x	2.903	2.04	4x	–
323	2x + 3x + 4x	2.903	2.04	4x	–
323	2x + 3x + 4x	2.903	2.04	4x	–
323	2x + 3x + 4x	2.903	2.04	4x	–
323	2x + 3x + 4x	2.903	1.16	4x	–
323	2x + 3x + 4x	2.905	2.03	4x	–
323	2x + 3x + 4x	2.908	1.94	4x	–
323	2x + 3x + 4x	2.908	2.73	4x	–
323	2x + 3x + 4x	2.909	1.73	4x	–
323	2x + 3x + 4x	2.909	1.56	4x	–
323	2x + 3x + 4x	2.909	3.23	4x	–
323	2x + 3x + 4x	2.909	3.21	4x	–
323	2x + 3x + 4x	2.910	2.07	4x	–
323	2x + 3x + 4x	2.910	2.45	4x	–
323	2x + 3x + 4x	2.910	2.43	4x	–
323	2x + 3x + 4x	2.911	2.80	4x	–
323	2x + 3x + 4x	2.911	2.66	4x	–
323	2x + 3x + 4x	2.911	2.66	4x	–
323	2x + 3x + 4x	2.911	2.66	4x	–
323	2x + 3x + 4x	2.911	2.66	4x	–
323	2x + 3x + 4x	2.911	2.66	4x	–
323	2x + 3x + 4x	2.911	1.89	4x	–
323	2x + 3x + 4x	2.911	1.89	4x	–
323	2x + 3x + 4x	2.911	1.89	4x	–
323	2x + 3x + 4x	2.911	1.89	4x	–
323	2x + 3x + 4x	2.911	1.89	4x	–
323	2x + 3x + 4x	2.911	1.68	4x	–
323	2x + 3x + 4x	2.911	3.30	4x	–
323	2x + 3x + 4x	2.911	3.30	4x	–
323	2x + 3x + 4x	2.911	3.30	4x	–
323	2x + 3x + 4x	2.911	3.30	4x	–
323	2x + 3x + 4x	2.911	2.04	4x	–
323	2x + 3x + 4x	2.911	2.04	4x	–
323	2x + 3x + 4x	2.911	2.04	4x	–
323	2x + 3x + 4x	2.911	2.04	4x	–
323	2x + 3x + 4x	2.911	1.90	4x	–
323	2x + 3x + 4x	2.911	1.90	4x	–
323	2x + 3x + 4x	2.911	1.90	4x	–
323	2x + 3x + 4x	2.911	1.90	4x	–
323	2x + 3x + 4x	2.911	1.67	4x	–
323	2x + 3x + 4x	2.911	1.67	4x	–
323	2x + 3x + 4x	2.911	1.67	4x	–

323	2x + 3x + 4x	2.911	1.67	4x	–
323	2x + 3x + 4x	2.911	1.67	4x	–
323	2x + 3x + 4x	2.911	1.67	4x	–
323	2x + 3x + 4x	2.911	1.67	4x	–
323	2x + 3x + 4x	2.912	1.79	4x	–
323	2x + 3x + 4x	2.912	1.79	4x	–
323	2x + 3x + 4x	2.912	1.79	4x	–
323	2x + 3x + 4x	2.912	1.79	4x	–
323	2x + 3x + 4x	2.912	1.79	4x	–
323	2x + 3x + 4x	2.912	1.79	4x	–
323	2x + 3x + 4x	2.913	1.53	4x	–
323	2x + 3x + 4x	2.913	1.53	4x	–
323	2x + 3x + 4x	2.913	1.53	4x	–
323	2x + 3x + 4x	2.916	2.04	4x	–
323	2x + 3x + 4x	2.918	3.60	4x	–
323	2x + 3x + 4x	2.918	2.20	4x	–
323	2x + 3x + 4x	2.918	2.20	4x	–
323	2x + 3x + 4x	2.918	2.20	4x	–
323	2x + 3x + 4x	2.918	2.20	4x	–
323	2x + 3x + 4x	2.918	2.20	4x	–
323	2x + 3x + 4x	2.918	2.20	4x	–
323	2x + 3x + 4x	2.919	1.30	4x	–
323	2x + 3x + 4x	2.919	1.97	4x	–
323	2x + 3x + 4x	2.922	2.79	4x	–
323	2x + 3x + 4x	2.922	2.79	4x	–
323	2x + 3x + 4x	2.922	2.79	4x	–
323	2x + 3x + 4x	2.922	2.79	4x	–
323	2x + 3x + 4x	2.922	2.79	4x	–
323	2x + 3x + 4x	2.923	2.87	4x	–
323	2x + 3x + 4x	2.924	3.04	4x	–
323	2x + 3x + 4x	2.924	3.04	4x	–
323	2x + 3x + 4x	2.924	3.04	4x	–
323	2x + 3x + 4x	2.924	3.04	4x	–
323	2x + 3x + 4x	2.925	2.15	4x	–
323	2x + 3x + 4x	2.925	2.15	4x	–
323	2x + 3x + 4x	2.925	2.15	4x	–
323	2x + 3x + 4x	2.925	2.15	4x	–
323	2x + 3x + 4x	2.925	2.15	4x	–
323	2x + 3x + 4x	2.925	2.15	4x	–
323	2x + 3x + 4x	2.929	2.39	4x	–
323	2x + 3x + 4x	2.929	2.39	4x	–
323	2x + 3x + 4x	2.929	2.39	4x	–
323	2x + 3x + 4x	2.929	2.39	4x	–
323	2x + 3x + 4x	2.929	2.39	4x	–
323	2x + 3x + 4x	2.930	3.03	4x	–
323	2x + 3x + 4x	2.931	3.24	4x	–
323	2x + 3x + 4x	2.931	3.24	4x	–
323	2x + 3x + 4x	2.931	3.24	4x	–
323	2x + 3x + 4x	2.931	3.24	4x	–
323	2x + 3x + 4x	2.931	3.24	4x	–
323	2x + 3x + 4x	2.931	3.24	4x	–
323	2x + 3x + 4x	2.931	3.50	4x	–
323	2x + 3x + 4x	2.931	1.99	4x	–
323	2x + 3x + 4x	2.931	1.99	4x	–
323	2x + 3x + 4x	2.931	1.99	4x	–
323	2x + 3x + 4x	2.931	1.99	4x	–
323	2x + 3x + 4x	2.931	1.99	4x	–
323	2x + 3x + 4x	2.931	1.99	4x	–
323	2x + 3x + 4x	2.931	2.72	4x	–
323	2x + 3x + 4x	2.931	2.69	4x	–
323	2x + 3x + 4x	2.931	2.69	4x	–
323	2x + 3x + 4x	2.931	2.69	4x	–
323	2x + 3x + 4x	2.931	2.69	4x	–

323	2x + 3x + 4x	2.931	2.69	4x	–
323	2x + 3x + 4x	2.931	2.69	4x	–
323	2x + 3x + 4x	2.931	1.58	4x	–
323	2x + 3x + 4x	2.931	1.58	4x	–
323	2x + 3x + 4x	2.931	1.58	4x	–
323	2x + 3x + 4x	2.931	1.58	4x	–
323	2x + 3x + 4x	2.931	1.58	4x	–
323	2x + 3x + 4x	2.931	1.58	4x	–
323	2x + 3x + 4x	2.931	1.24	4x	–
323	2x + 3x + 4x	2.931	1.37	4x	–
323	2x + 3x + 4x	2.931	1.20	4x	–
323	2x + 3x + 4x	2.931	1.14	4x	–
323	2x + 3x + 4x	2.933	1.79	4x	–
323	2x + 3x + 4x	2.935	1.65	4x	–
323	2x + 3x + 4x	2.935	1.65	4x	–
323	2x + 3x + 4x	2.935	1.65	4x	–
323	2x + 3x + 4x	2.935	1.65	4x	–
323	2x + 3x + 4x	2.935	1.65	4x	–
323	2x + 3x + 4x	2.935	1.65	4x	–
323	2x + 3x + 4x	2.938	1.73	4x	–
323	2x + 3x + 4x	2.939	2.28	4x	–
323	2x + 3x + 4x	2.939	2.28	4x	–
323	2x + 3x + 4x	2.939	2.28	4x	–
323	2x + 3x + 4x	2.939	2.28	4x	–
323	2x + 3x + 4x	2.939	2.28	4x	–
323	2x + 3x + 4x	2.939	1.55	4x	–
323	2x + 3x + 4x	2.939	1.73	4x	–
323	2x + 3x + 4x	2.939	1.73	4x	–
323	2x + 3x + 4x	2.941	3.51	4x	–
323	2x + 3x + 4x	2.941	2.20	4x	–
323	2x + 3x + 4x	2.941	1.76	4x	–
323	2x + 3x + 4x	2.941	1.76	4x	–
323	2x + 3x + 4x	2.941	1.76	4x	–
323	2x + 3x + 4x	2.941	1.76	4x	–
323	2x + 3x + 4x	2.941	1.76	4x	–
323	2x + 3x + 4x	2.942	1.40	4x	–
323	2x + 3x + 4x	2.942	1.46	4x	–
323	2x + 3x + 4x	2.942	1.46	4x	–
323	2x + 3x + 4x	2.942	1.46	4x	–
323	2x + 3x + 4x	2.942	1.46	4x	–
323	2x + 3x + 4x	2.942	1.46	4x	–
323	2x + 3x + 4x	2.942	1.46	4x	–
323	2x + 3x + 4x	2.942	1.27	4x	–
323	2x + 3x + 4x	2.942	1.27	4x	–
323	2x + 3x + 4x	2.942	1.27	4x	–
323	2x + 3x + 4x	2.942	1.27	4x	–
323	2x + 3x + 4x	2.942	1.27	4x	–
323	2x + 3x + 4x	2.942	2.40	4x	–
323	2x + 3x + 4x	2.942	2.40	4x	–
323	2x + 3x + 4x	2.942	2.40	4x	–
323	2x + 3x + 4x	2.942	2.40	4x	–
323	2x + 3x + 4x	2.942	2.40	4x	–
323	2x + 3x + 4x	2.942	2.40	4x	–
323	2x + 3x + 4x	2.943	2.07	4x	–
323	2x + 3x + 4x	2.943	2.07	4x	–
323	2x + 3x + 4x	2.943	2.07	4x	–
323	2x + 3x + 4x	2.943	2.07	4x	–
323	2x + 3x + 4x	2.943	2.07	4x	–
323	2x + 3x + 4x	2.943	2.07	4x	–
323	2x + 3x + 4x	2.943	2.07	4x	–
323	2x + 3x + 4x	2.949	3.47	4x	–
323	2x + 3x + 4x	2.949	3.47	4x	–
323	2x + 3x + 4x	2.949	3.47	4x	–

323	2x + 3x + 4x	2.950	3.46	4x	–
323	2x + 3x + 4x	2.950	1.91	4x	–
323	2x + 3x + 4x	2.950	1.91	4x	–
323	2x + 3x + 4x	2.950	1.91	4x	–
323	2x + 3x + 4x	2.950	1.91	4x	–
323	2x + 3x + 4x	2.950	1.91	4x	–
323	2x + 3x + 4x	2.950	2.20	4x	–
323	2x + 3x + 4x	2.950	2.20	4x	–
323	2x + 3x + 4x	2.950	2.20	4x	–
323	2x + 3x + 4x	2.950	2.20	4x	–
323	2x + 3x + 4x	2.950	3.02	4x	–
323	2x + 3x + 4x	2.950	1.73	4x	–
323	2x + 3x + 4x	2.950	1.73	4x	–
323	2x + 3x + 4x	2.950	1.73	4x	–
323	2x + 3x + 4x	2.950	1.73	4x	–
323	2x + 3x + 4x	2.950	1.73	4x	–
323	2x + 3x + 4x	2.950	1.42	4x	–
323	2x + 3x + 4x	2.950	1.42	4x	–
323	2x + 3x + 4x	2.951	1.30	4x	–
323	2x + 3x + 4x	2.957	2.15	4x	–
323	2x + 3x + 4x	2.958	2.83	4x	–
323	2x + 3x + 4x	2.958	2.83	4x	–
323	2x + 3x + 4x	2.960	3.75	4x	–
323	2x + 3x + 4x	2.960	3.75	4x	–
323	2x + 3x + 4x	2.960	3.75	4x	–
323	2x + 3x + 4x	2.960	3.75	4x	–
323	2x + 3x + 4x	2.960	3.75	4x	–
323	2x + 3x + 4x	2.960	3.75	4x	–
323	2x + 3x + 4x	2.960	3.31	4x	–
323	2x + 3x + 4x	2.960	2.64	4x	–
323	2x + 3x + 4x	2.960	2.64	4x	–
323	2x + 3x + 4x	2.960	2.64	4x	–
323	2x + 3x + 4x	2.960	2.64	4x	–
323	2x + 3x + 4x	2.960	2.64	4x	–
323	2x + 3x + 4x	2.960	2.64	4x	–
323	2x + 3x + 4x	2.960	2.64	4x	–
323	2x + 3x + 4x	2.962	2.69	4x	–
323	2x + 3x + 4x	2.962	2.69	4x	–
323	2x + 3x + 4x	2.962	2.69	4x	–
323	2x + 3x + 4x	2.969	2.44	4x	–
323	2x + 3x + 4x	2.969	2.44	4x	–
323	2x + 3x + 4x	2.969	2.44	4x	–
323	2x + 3x + 4x	2.969	2.44	4x	–
323	2x + 3x + 4x	2.970	2.18	4x	–
323	2x + 3x + 4x	2.970	2.18	4x	–
323	2x + 3x + 4x	2.970	2.04	4x	–
323	2x + 3x + 4x	2.970	1.77	4x	–
323	2x + 3x + 4x	2.970	1.68	4x	–
323	2x + 3x + 4x	2.970	1.68	4x	–
323	2x + 3x + 4x	2.971	1.84	4x	–
323	2x + 3x + 4x	2.971	1.84	4x	–
323	2x + 3x + 4x	2.971	1.95	4x	–
323	2x + 3x + 4x	2.971	1.95	4x	–
323	2x + 3x + 4x	2.971	1.95	4x	–
323	2x + 3x + 4x	2.971	1.95	4x	–
323	2x + 3x + 4x	2.971	1.95	4x	–
323	2x + 3x + 4x	2.971	2.61	4x	–
323	2x + 3x + 4x	2.971	2.61	4x	–
323	2x + 3x + 4x	2.971	2.61	4x	–
323	2x + 3x + 4x	2.971	1.45	4x	–
323	2x + 3x + 4x	2.971	1.45	4x	–
323	2x + 3x + 4x	2.971	1.45	4x	–
323	2x + 3x + 4x	2.971	1.45	4x	–

323	2x + 3x + 4x	2.971	1.45	4x	–
323	2x + 3x + 4x	2.971	2.59	4x	–
323	2x + 3x + 4x	2.971	2.59	4x	–
323	2x + 3x + 4x	2.971	2.59	4x	–
323	2x + 3x + 4x	2.979	3.26	4x	–
323	2x + 3x + 4x	2.979	3.26	4x	–
323	2x + 3x + 4x	2.979	3.26	4x	–
323	2x + 3x + 4x	2.979	3.26	4x	–
323	2x + 3x + 4x	2.979	3.26	4x	–
323	2x + 3x + 4x	2.979	3.26	4x	–
323	2x + 3x + 4x	2.979	3.26	4x	–
323	2x + 3x + 4x	2.980	1.84	4x	–
323	2x + 3x + 4x	2.980	2.24	4x	–
323	2x + 3x + 4x	2.980	2.24	4x	–
323	2x + 3x + 4x	2.980	2.24	4x	–
323	2x + 3x + 4x	2.980	2.24	4x	–
323	2x + 3x + 4x	2.980	2.24	4x	–
323	2x + 3x + 4x	2.980	2.79	4x	–
323	2x + 3x + 4x	2.980	1.67	4x	–
323	2x + 3x + 4x	2.980	1.67	4x	–
323	2x + 3x + 4x	2.990	3.18	4x	–
323	2x + 3x + 4x	2.990	3.18	4x	–
323	2x + 3x + 4x	2.990	3.18	4x	–
323	2x + 3x + 4x	2.990	3.22	4x	–
323	2x + 3x + 4x	2.990	3.22	4x	–
323	2x + 3x + 4x	2.990	3.22	4x	–
323	2x + 3x + 4x	2.990	3.22	4x	–
323	2x + 3x + 4x	2.990	3.22	4x	–
323	2x + 3x + 4x	2.990	2.37	4x	–
323	2x + 3x + 4x	2.990	2.37	4x	–
323	2x + 3x + 4x	2.990	2.37	4x	–
323	2x + 3x + 4x	2.990	2.37	4x	–
323	2x + 3x + 4x	2.990	2.37	4x	–
323	2x + 3x + 4x	2.990	2.91	4x	–
323	2x + 3x + 4x	2.990	2.91	4x	–
323	2x + 3x + 4x	2.990	2.91	4x	–
323	2x + 3x + 4x	2.990	2.91	4x	–
323	2x + 3x + 4x	2.990	2.91	4x	–
323	2x + 3x + 4x	2.990	3.54	4x	–
323	2x + 3x + 4x	2.990	3.54	4x	–
323	2x + 3x + 4x	2.990	3.54	4x	–
323	2x + 3x + 4x	3.000	2.49	4x	–
323	2x + 3x + 4x	3.000	2.49	4x	–
323	2x + 3x + 4x	3.000	2.49	4x	–
323	2x + 3x + 4x	3.000	2.49	4x	–
323	2x + 3x + 4x	3.000	2.49	4x	–
323	2x + 3x + 4x	3.000	3.62	4x	–
323	2x + 3x + 4x	3.000	3.62	4x	–
323	2x + 3x + 4x	3.000	3.62	4x	–
323	2x + 3x + 4x	3.000	3.62	4x	–
323	2x + 3x + 4x	3.000	1.68	4x	–
323	2x + 3x + 4x	3.000	4.05	4x	–
323	2x + 3x + 4x	3.000	4.05	4x	–
323	2x + 3x + 4x	3.000	4.05	4x	–
323	2x + 3x + 4x	3.000	4.05	4x	–
323	2x + 3x + 4x	3.000	4.05	4x	–
323	2x + 3x + 4x	3.000	3.13	4x	–
323	2x + 3x + 4x	3.000	3.13	4x	–
323	2x + 3x + 4x	3.000	3.13	4x	–
323	2x + 3x + 4x	3.000	3.13	4x	–
323	2x + 3x + 4x	3.000	3.13	4x	–
323	2x + 3x + 4x	3.010	1.79	4x	–
323	2x + 3x + 4x	3.010	2.81	4x	–

323	2x + 3x + 4x	3.010	2.81	4x	–
323	2x + 3x + 4x	3.010	2.81	4x	–
323	2x + 3x + 4x	3.010	3.35	4x	–
323	2x + 3x + 4x	3.010	3.35	4x	–
323	2x + 3x + 4x	3.010	3.35	4x	–
323	2x + 3x + 4x	3.010	3.35	4x	–
323	2x + 3x + 4x	3.010	3.62	4x	–
323	2x + 3x + 4x	3.010	3.62	4x	–
323	2x + 3x + 4x	3.010	3.62	4x	–
323	2x + 3x + 4x	3.010	3.62	4x	–
323	2x + 3x + 4x	3.010	3.45	4x	–
323	2x + 3x + 4x	3.010	3.45	4x	–
323	2x + 3x + 4x	3.010	3.45	4x	–
323	2x + 3x + 4x	3.010	3.45	4x	–
323	2x + 3x + 4x	3.010	3.06	4x	–
323	2x + 3x + 4x	3.010	3.06	4x	–
323	2x + 3x + 4x	3.010	3.06	4x	–
323	2x + 3x + 4x	3.010	3.06	4x	–
323	2x + 3x + 4x	3.010	3.06	4x	–
323	2x + 3x + 4x	3.010	3.06	4x	–
323	2x + 3x + 4x	3.010	3.62	4x	–
323	2x + 3x + 4x	3.010	3.62	4x	–
323	2x + 3x + 4x	3.010	3.62	4x	–
323	2x + 3x + 4x	3.010	3.62	4x	–
323	2x + 3x + 4x	3.020	1.84	4x	–
323	2x + 3x + 4x	3.021	3.27	4x	–
323	2x + 3x + 4x	3.021	3.27	4x	–
323	2x + 3x + 4x	3.021	3.27	4x	–
323	2x + 3x + 4x	3.021	3.27	4x	–
323	2x + 3x + 4x	3.021	3.27	4x	–
323	2x + 3x + 4x	3.030	2.46	4x	–
323	2x + 3x + 4x	3.033	2.51	4x	–
323	2x + 3x + 4x	3.033	2.51	4x	–
323	2x + 3x + 4x	3.033	2.51	4x	–
323	2x + 3x + 4x	3.040	3.51	4x	–
323	2x + 3x + 4x	3.040	3.51	4x	–
323	2x + 3x + 4x	3.040	3.51	4x	–
323	2x + 3x + 4x	3.040	3.51	4x	–
323	2x + 3x + 4x	3.040	3.51	4x	–
323	2x + 3x + 4x	3.041	3.36	4x	–
323	2x + 3x + 4x	3.041	3.36	4x	–
323	2x + 3x + 4x	3.041	3.36	4x	–
323	2x + 3x + 4x	3.041	3.36	4x	–
323	2x + 3x + 4x	3.050	3.28	4x	–
323	2x + 3x + 4x	3.050	3.28	4x	–
323	2x + 3x + 4x	3.050	3.28	4x	–
323	2x + 3x + 4x	3.050	3.28	4x	–
323	2x + 3x + 4x	3.050	3.28	4x	–
323	2x + 3x + 4x	3.053	2.97	4x	–
323	2x + 3x + 4x	3.053	2.97	4x	–
323	2x + 3x + 4x	3.053	2.97	4x	–
323	2x + 3x + 4x	3.053	2.97	4x	–
323	2x + 3x + 4x	3.061	2.31	4x	–
323	2x + 3x + 4x	3.062	4.08	4x	–
323	2x + 3x + 4x	3.063	3.47	4x	–
323	2x + 3x + 4x	3.064	3.52	4x	–
323	2x + 3x + 4x	3.064	3.42	4x	–
323	2x + 3x + 4x	3.064	3.42	4x	–
323	2x + 3x + 4x	3.064	3.42	4x	–
323	2x + 3x + 4x	3.064	3.42	4x	–
323	2x + 3x + 4x	3.064	3.42	4x	–
323	2x + 3x + 4x	3.071	1.99	4x	–
323	2x + 3x + 4x	3.106	2.39	4x-5x	2n = 4x+1 = 37

323	2x + 3x + 4x	3.163	1.33	4x-5x	2n = 4x+3 = 39
323	2x + 3x + 4x	3.177	3.10	4x-5x	–
323	2x + 3x + 4x	3.196	2.15	4x-5x	–
323	2x + 3x + 4x	3.204	1.68	4x-5x	–
323	2x + 3x + 4x	3.210	1.41	4x-5x	2n = 4x+4 = 40
323	2x + 3x + 4x	3.220	1.09	4x-5x	–
323	2x + 3x + 4x	3.265	1.82	4x-5x	–
323	2x + 3x + 4x	3.333	1.92	4x-5x	–
323	2x + 3x + 4x	3.340	1.88	4x-5x	–
323	2x + 3x + 4x	3.439	1.74	4x-5x	–
323	2x + 3x + 4x	3.505	1.11	4x-5x	–
323	2x + 3x + 4x	4.119	1.39	6x	–
323	2x + 3x + 4x	4.232	2.10	6x	–
323	2x + 3x + 4x	4.433	1.48	6x	–

[*] The numbers correspond to population codes in the complete list of populations under study (Supplementary Data Table S1).

[†] Relative genome size is presented as a ratio of peak of a sample to that of an internal standard based on FCM histograms of fluorescence intensity.

[‡] A coefficient of variation of the sample G_0/G_1 peak expressing the error of measurement.

PAPER IV

Climatic conditions and human activities shape diploid – tetraploid coexistence at different spatial scales in the common weed *Tripleurospermum inodorum* (Asteraceae)

A population of *Tripleurospermum inodorum* dominating the vegetation of a small fallow in between fields.

Climatic conditions and human activities shape diploid – tetraploid coexistence at different spatial scales in the common weed *Tripleurospermum inodorum* (Asteraceae)

Martin Čertner[1,2,5], Pavel Kúr[3], Filip Kolář[1,2,4] & Jan Suda[1,2]

[1]Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, CZ-128 00 Prague, Czech Republic; [2]Institute of Botany, The Czech Academy of Sciences, Zámek 1, CZ-252 43 Průhonice, Czech Republic; [3]Department of Botany, National Museum, Cirkusová 1740, CZ-193 00 Prague, Czech Republic; and [4]National Centre for Biosystematics, Natural History Museum, University of Oslo, NO-0318 Oslo, Norway

[5]Author for correspondence (e-mail: martin.certner@gmail.com)

ABSTRACT

Premise of the study: Environmental conditions are important determinants of cytotype distribution patterns in some mixed-ploidy species. Because the ecology of cytotypes is usually compared at one selected spatial scale, possible involvement of several environmental factors operating at distinct scales may remain undetected. We adopted several complementary approaches to assess ecological requirements of cytotypes across a wide-range of spatial scales.

Methods: WorldClim extracted data were used for comparing climatic niches of *Tripleurospermum inodorum* diploids and tetraploids in (Central) Europe and predicting climatic suitability of habitats in Europe. Small-scale ecological preferences of cytotypes were assessed independently in three regions within the contact zone. Habitat types and ecological indicators inferred from species composition of vegetation in both uniform-ploidy and mixed-ploidy populations were used for ecological niche reconstructions.

Key results: The two cytotypes differed in their climatic niches, however, due to a considerable niche overlap, diploids and tetraploids were predicted to co-occur in an extensive contact zone in Europe. Within the contact zone, cytotypes had identical ecological preferences but varied in their relative occurrence in particular habitat types. The ecological niches of mixed-ploidy and uniform-ploidy populations were undistinguishable.

Conclusions: Climate acts as an important driver of cytotype distribution patterns at larger spatial scales, delimiting the position and breath of the contact zone. However, within the contact zone, at regional and finer scales, non-random migration patterns caused by human activities and the founder effect seem to be more important than environmental heterogeneity. No ecological constraints to cytotype coexistence were detected.

Key words: contact zone, cytotype coexistence, ecological indicator values, ecological niche modelling, habitat preferences, *Matricaria perforata*, polyploidy, scentless mayweed.

INTRODUCTION

Polyploidy, the possession of more than two complete sets of chromosomes in the nucleus, have fascinated botanists for over a century (Ramsey and Ramsey, 2014). Recent studies suggest that all angiosperms have underwent one or more polyploidization events in their evolutionary history (Wendel et al., 2016), and this process even triggered rapid diversification in some angiosperm lineages (Soltis et al., 2009). The evolutionary significance of polyploidy stems mainly from an instantaneous reproductive isolation from diploid progenitors, which makes it an efficient mechanism of sympatric speciation (Coyne and Orr, 2004; Wood et al., 2009) and from the fact that polyploidization often brings genetic and phenotypic novelty (Levin, 2002; Flagel and Wendel, 2009; Hegarty et al., 2013).

Nonetheless, immediately after their origin, new polyploids are facing strong minority disadvantage due to reproductive interactions with diploid progenitors (Husband, 2000), which may ultimately lead to their local extinction (Levin, 1975). The chances of successful polyploid establishment may be elevated if the pollen transfer is non-random and within-ploidy mating is favored (due to e.g. flowering-time shifts, pollinator preferences, and spatial segregation of cytotypes within populations; Segraves and Thompson, 1999; Nuismer and Cunningham, 2005; Trávníček et al., 2011) or, alternatively, if the polyploid has a mechanism of reproductive assurance (e.g. autogamy, clonal reproduction; Barringer, 2007; Kirchheimer et al., 2016). Frequently observed contrasting ecogeographical distributions of closely related diploids and polyploids (reviewed in Ehrendorfer, 1980; Levin, 2002) led to a common belief that phenotypic novelty, arising as a direct consequence of polyploidization, pre-adapts polyploids for colonizing new ecological niches (Ramsey and Ramsey, 2014). According to theoretical models, ploidy-specific differences in survival and/or fitness under particular environmental conditions could lead to spatial segregation of cytotypes in environmentally heterogeneous landscapes and thus allow both polyploid establishment and subsequent cytotype coexistence (Fowler and Levin, 1984; Rodríguez, 1996). Habitat segregation of cytotypes was repeatedly reported, either as a mechanism allowing the establishment of young polyploid derivatives in populations of their lower-ploid progenitors (Lumaret et al., 1987; Ramsey, 2011) or, as a way of facilitating the coexistence of already established cytotypes encountering each other in secondary contact zones (e.g. Manzaneda et al., 2012; Laport et al., 2013; Sonnleitner et al., 2016).

On the other hand, the lack of habitat segregation of cytotypes observed in many other studies (e.g. Baack and Stanton, 2005; Buggs and Pannell, 2007; Hanzl et al., 2014) along with the absence of consistent climatic niche shifts in a comprehensive set of 20 conspecific diploid – young polyploid comparisons (Glennon et al., 2014) suggest that ecological segregation of cytotypes may rather be an occasional scenario than the rule. When identifying the cases of ecological segregation of cytotypes, different authors relied on different methods and employed for instance assessments of (macro-)climatic conditions, habitat-type preferences, vegetation composition, and local environmental parameters (either directly measured in the field or reconstructed from the species composition using ecological indicator values). The different methodology not only complicates across-species generalization but each method is typically applied at a different spatial scale. Although one might expect that cytotype distribution patterns could result from an interplay of several environmental factors operating at distinct spatial scales (e.g. large-scale effect of climate, small-scale effect of soil quality), complex assessments of habitat differentiation of cytotypes across several spatial scales have only exceptionally been applied (Treier et al., 2009; Laport et al., 2013; Kirchheimer et al., 2016).

In this study, we aim at comprehensive assessment of habitat requirements of cytotypes of a single species across a wide range of spatial scales (from population level to continental) by using several complementary approaches. Our model plant is scentless mayweed (*Tripleurospermum inodorum*), a very common and widespread weed occurring under a wide range of climatic and ecological conditions in Europe (Kay, 1994). Two major cytotypes of the species, diploids and tetraploids, prevail in the western and eastern parts of the continent, respectively (Kay, 1969). In Central Europe, both diploids and tetraploids coexist in a diffuse contact zone and spatial trends in cytotype distribution were well-mapped recently (Čertner et al., 2017). Specifically, we ask the following questions: (1) Is the distribution of *T. inodorum* diploids and tetraploids in Europe driven by climatic conditions? If so, where can we predict uniform-ploidy areas and where co-occurrence of the two cytotypes? (2) Could climatic conditions contribute to spatial segregation of cytotypes even in the zone of sympatry? (3) Do diploids and tetraploids in the contact zone differ in their preferences for habitat type and/or local ecological conditions? (4) What environmental conditions favor the local cytotype coexistence? Are mixed-ploidy populations bound to intermediate niches, marginal habitats, sites with higher microhabitat heterogeneity and/or disturbance frequency?

MATERIALS AND METHODS

Study organism

Scentless mayweed [*Tripleurospermum inodorum* (L.) Sch. Bip., syn. *Matricaria perforata* Mérat, Asteraceae] is a common annual weed of fields and other man-disturbed sites (Kubát, 2004). The plants depend solely on sexual reproduction and following insect pollination, they produce thousands of achenes that lack any obvious adaptations for long-distance dispersal. Its achenes can survive for at least ten years buried in soil and develop a permanent soil seed bank (Kay, 1994).

Tripleurospermum inodorum is considered native to most parts of Europe and Western Asia and has also been introduced to North America and some other temperate regions (Kay, 1976, 1994). Two morphologically indistinguishable cytotypes, diploid (2n = 18) and tetraploid (2n = 36), have been reported from both the native and the introduced ranges (Kay, 1969; Woo et al., 1991). In Europe, diploids prevail in western parts of the continent whereas tetraploids dominate in the east, and both cytotypes co-occur in Central Europe (Kay, 1969). Diploid – tetraploid contact zone is wide and has a diffuse, mosaic-like character with very common incidence of mixed-ploidy populations in some regions (up to 43%; Čertner et al., 2017). Substantial differences in monoploid genome sizes between the two cytotypes suggest that *T. inodorum* tetraploids either underwent substantial diversification following their origin or that they originated from a diploid lineage other than the one they are currently sympatric with (Čertner et al., 2017). That clearly demonstrates secondary origin of the cytotype contact (*sensu* Petit et al., 1999).

Climatic conditions

For comparison of climatic niches of diploids and tetraploids, we compiled a set of 1 381 georeferenced occurrences of *T. inodorum* cytotypes across Europe. It consisted of 1 209 populations sampled in our previous study (Čertner et al., 2017), 128 literature records (Kay, 1969; Lankosz-Mróz, 1976), and of our 44 previously unpublished records (Appendix S1; see Supplemental Data with the online version of this article). Two separate datasets were prepared for comparisons at the European and Central-European scales, respectively (Fig. 1). To account for a strong heterogeneity in sampling intensity, we divided the study areas on a grid of equal-sized cells (Europe: 35 × 35 km, Central Europe: 17 × 17 km) and, when possible, we randomly selected one diploid and one tetraploid occurrence per cell (in either uniform-ploidy or mixed-ploidy population, this choice was also random). The reduced datasets consisted of 110 diploid + 184 tetraploid localities and 75 diploid + 257 tetraploid localities, at the European and Central-European scales, respectively. Georeferenced occurrences of both cytotypes were used to extract climate data from 19 BIOCLIM variables of the WorldClim database (http://www.worldclim.org/bioclim; Hijmans et al., 2005) downloaded in the highest available resolution (30 arc seconds ≈ 1 km^2) using ArcGIS 10.0 (ESRI, Redlands, California, USA).

Habitat types and vegetation samples

Habitat preferences of cytotypes were assessed independently in three regions of common cytotype co-occurrence located in the Czech Republic (Fig. 1): MAR (30 × 30 km; coordinates of the centroid: 49°58'28"N, 12°45'29"E), RAK (28 × 31 km; 50°9'59"N, 13°43'15"E), and SOB (21 × 23 km; 49°16'59"N, 14°52'13"E). We adopted two complementary approaches for the task. Firstly, the occurrence of diploid, tetraploid, and mixed-ploidy *T. inodorum* populations at various habitat types within the three study regions was extracted from the sampling information of our

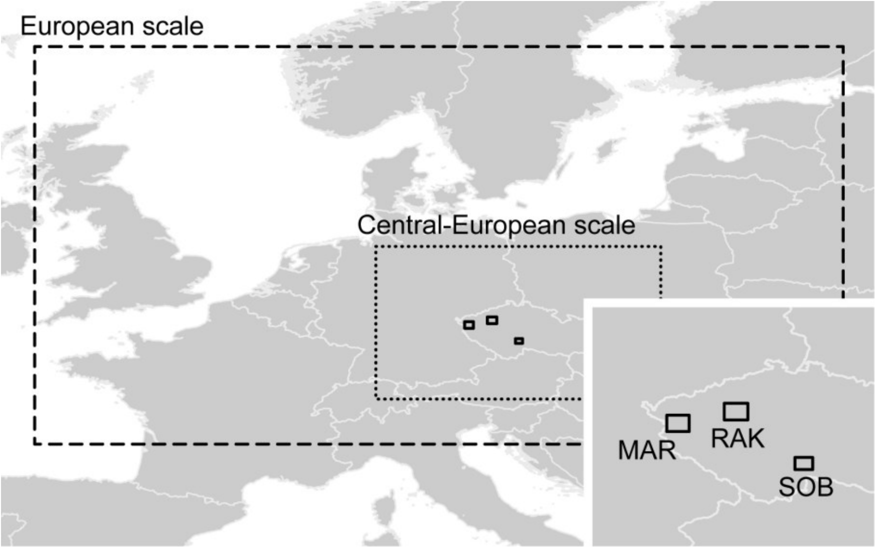

Fig. 1. The study areas of environmental niche comparisons between diploids and tetraploids of *T. inodorum*. Climatic niches of the cytotypes were contrasted at the European scale (dashed line), capturing both uniform-ploidy and mixed-ploidy parts of the distributional range, as well as at the Central-European scale (dotted line) targeted at their diffuse contact zone. Habitat and vegetation assessments were conducted independently in three regions of the Czech Republic with common co-occurrence of the two cytotypes (MAR, RAK, SOB).

previous study (Čertner et al., 2017). Secondly, vegetation samples were recorded at localities inhabited either by uniform-ploidy or mixed-ploidy populations within each of the three regions. The particular localities were selected in order to i) sample representatively diploid, tetraploid and mixed-ploidy populations, ii) capture regional habitat diversity, and iii) to cover the most part of the study regions. When two or more close-by localities (< 1.5 km) shared their cytotype composition and habitat type, one of them was randomly selected. Vegetation samples were recorded in 50, 35, and 38 populations from the regions MAR, RAK, and SOB, respectively (Appendix S2; see Supplemental Data with the online version of this article).

At each site, vegetation was documented in circular plots of 0.3 m radius centered at each of five randomly selected *T. inodorum* individuals (Appendix S3; see Supplemental Data with the online version of this article). Within each plot, i) all vascular plant species were recorded, ii) a percentage cover of herb-layer vegetation was assessed, and iii) leaf samples of all *T. inodorum* individuals present there were collected for ploidy estimation. Ploidy level was inferred from fluorescence intensities of DAPI-stained nuclei using flow cytometry, following the protocol used in our earlier study (Čertner et al., 2017). Though we were using data from our previous ploidy screening for the selection of suitable localities, population dynamics of *T. inodorum* mostly prevented sampling at exactly the same sites. Diploid, tetraploid and mixed-ploidy populations were thus delimited based on the ploidy of individuals found within the five vegetation samples. The presence of two or more cytotypes (incl. triploid hybrids) at a site classified it as a mixed-ploidy population. All vegetation samples were recorded from July to August 2014 to maximize their comparability.

Statistical analyses

Eleven habitat types extracted from the sampling information of our previous study (Čertner et al., 2017) were either merged into one of four arbitrary categories of main habitats (i.e. arable land, roadsides, ruderal sites, and grasslands) or excluded as clearly distinct and uncommon (e.g. river banks, unpaved forest roads). In total, 575 out of 610 *T. inodorum* population records could be reliably assigned into one of the four habitat categories. The relative occurrence of diploid, tetraploid and mixed-ploidy populations across the habitat categories was tested using Chi-square tests. Analyses were done separately for each of the three study regions. The Bonferroni correction was applied for pairwise comparisons between populations of different cytotype composition. An overall test of interactions among the three factors (i.e. cytotype composition of populations, habitat type, and study region) was done using generalized linear models with Poisson distribution (i.e. log-linear models) followed by sequential likelihood-ratio tests.

For unbiased comparisons of ecological niches of *T. inodorum* cytotypes, both within and among the three study regions, we avoided direct comparisons of floristic composition of the sampled plots. Instead, we used Ellenberg indicator values (EIVs) which provide estimates of ecological characteristics of the sites inferred from species composition data (Ellenberg et al., 2001). To each vegetation sample, we assigned mean EIVs for light (L), temperature (T), continentality (K), humidity (F), soil reaction (R), nutrients (N), and salinity (S). The ecological characteristics were complemented with species richness of each vegetation sample, relative coverage of the herb layer, and with mean values of disturbance frequency and disturbance severity indexes at a whole-community level (Herben et al., 2016) that were also reconstructed from the species composition. As each locality was represented by five replicate vegetation samples, we calculated mean values and standard deviations of all ecological parameters per locality and used them in all the downstream analyses. The standard deviations of parameters were intended as proxies of microhabitat heterogeneity. A principal component analysis (PCA) was employed to visualize ecological niches of diploid, tetraploid and mixed-ploidy populations separately in each region and across all the regions. Differences in ecological niches of cytotypes were tested using a redundancy analysis (RDA) on the complete dataset and within particular regions, with either cytotype composition of populations (diploid, tetraploid and mixed-ploidy) or proportion of diploids at the site as explanatory variables. Trends among the study regions were tested as an interaction between region and cytotype composition of populations in RDA.

A canonical discriminant analysis (CDA) was applied to i) compare climatic niches of the two cytotypes and ii) identify BIOCLIM variables with the highest power to predict cytotype distributions. Prior the analysis, we checked for normal distribution of climatic variables and randomly excluded one of each pair of strongly correlated variables (i.e. with absolute values of correlation coefficients >0.95). The final datasets consisted of 15 and 12 BIOCLIM variables for the European and Central-European scales, respectively.

All univariate analyses were conducted in R 3.3.2 (R Core Team, 2016) and multivariate analyses in Canoco 5 (Lepš and Šmilauer, 2014). Statistical significance of multivariate models was tested using a Monte Carlo test with 9 999 permutations.

Climatic niche models

For predicting the occurrence of *T. inodorum* cytotypes in Europe and identifying any climate-driven limits of their distributional ranges, we employed maximum entropy modeling approach implemented in MaxEnt 3.3.3k (Phillips and Dudík, 2008). We used the European-scale set of 294 diploid and tetraploid occurrences (see above) and the same 15 BIOCLIM variables previously used in CDA. Niche models for each cytotype were run with ten replicates and averaged values of estimates were used. Before each model run, the occurrences were randomly allocated for model building (75% of data) and for testing the model performance (25% of data) using the subsampling replication method. Cumulative models giving the probability of occurrence of a given cytotype at a particular site were generated using a 1 000 threshold for maximum number of iterations and including a bias file, the remaining model parameters were kept at default setting. The bias file was used to account for incomplete sampling by excluding non-sampled regions from model building and thus improving the model performance (Phillips et al., 2009). Because *T. inodorum* is very common in the most part of Europe, a conservative approach of creating polygons around thoroughly-sampled regions and 5-km buffers around more isolated localities was used as a proxy of sampling area. All 294 species occurrences (i.e. irrespective of a cytotype) were considered for constructing a bias file. The predictive power of climatic niche models was assessed using the value of "area under the receiver operating characteristic curve" (AUC; Fielding and Bell, 1997) and from the rates of average omission on test data points.

RESULTS

Climatic niche differentiation

Climatic niches of *T. inodorum* cytotypes were compared using two datasets: one capturing both uniform-ploidy and mixed-ploidy parts of *T. inodorum* distributional range (i.e. the European scale) and the other targeted at diffuse contact zone of the two cytotypes (i.e. the Central-European scale; Fig. 1). Significant differences in climatic niches of diploids and tetraploids were discovered at the both European and Central-European scales (canonical discriminant analysis, CDA; P = 0.0001 and P = 0.0003, respectively). While at the European scale the CDA identified climatic niches dominated by either of the two cytotypes as well as climatic niches shared by both of them (Fig. 2A), at the Central-European scale only tetraploid-dominated and shared niches were recognized (Fig. 2B). At the both spatial scales, *T. inodorum* diploids preferred regions with lower temperature seasonality and lower mean diurnal range of temperatures. The climatically suitable regions were further characterized at the European scale by lower mean temperature of the wettest quarter, higher mean temperature of the driest quarter, and higher mean temperature of the coldest quarter, whereas at the Central-European scale by lower temperature annual range and lower maximal temperature of the warmest month (Tab. 1).

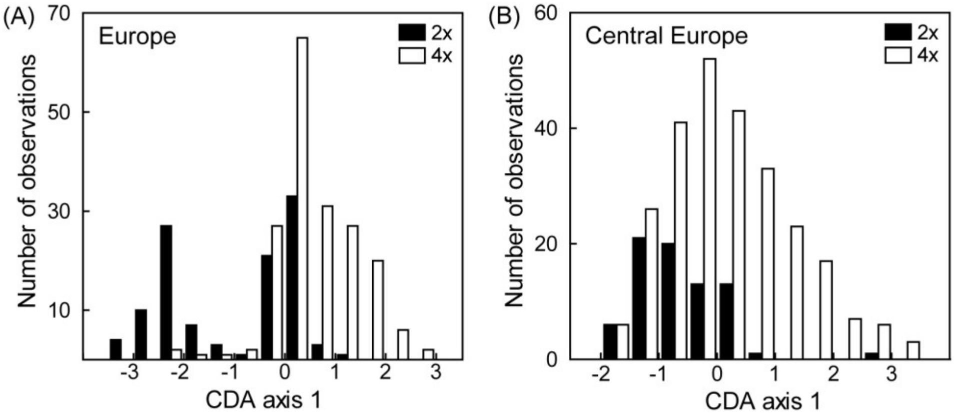

Fig. 2. Overlap between climatic niches of diploids and tetraploids at the European (A) and the Central-European (B) scales showed by a canonical discriminant analysis.

A maximum entropy modeling approach was used for predicting the climatic suitability of habitats for diploids and tetraploids at the European scale (Fig. 3). The highest probability of diploid occurrence was in western and northwestern Europe, from where it gradually decreased towards Central Europe. Further to the east were diploids likely to occur only in coastal regions and in higher altitudes (e.g. along the Baltic Sea and in the Carpathian mountain range). Tetraploids, on the other hand, were predicted to occur in most part of the modelled area except the westernmost regions and higher altitudes (i.e. the British Isles, coasts of France and Norway, Alps and Carpathians). Coexistence of the two cytotypes is thus likely in an extensive zone stretching from northern Italy to southern coasts of Norway and Sweden, and from central France to the Czech Republic and Baltic coast.

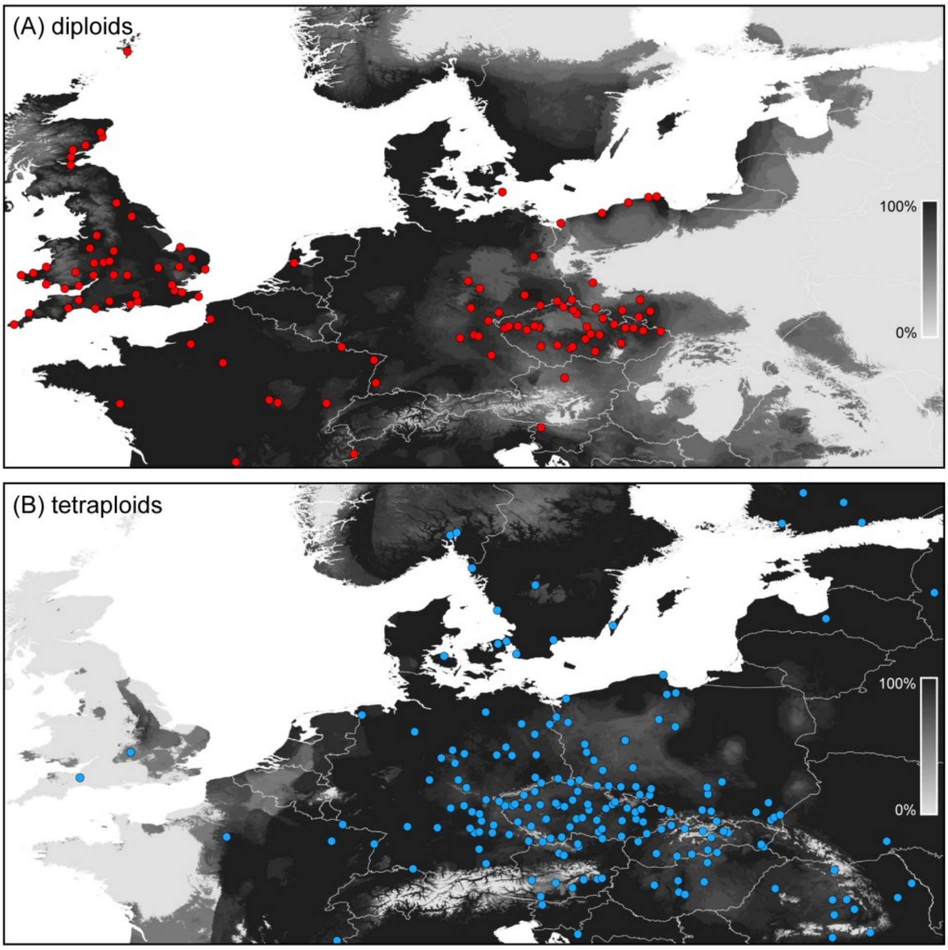

Fig. 3. Predicted climatic suitability of habitats for diploid (A) and tetraploid (B) cytotype of *T. inodorum* across Europe inferred by maximum entropy niche modelling. The darker the color, the higher the probability of cytotype occurrence in a given region. Points indicate diploid (red) and tetraploid (blue) occurrences used for model building and testing.

For both diploids and tetraploids, the model testing procedures resulted in sufficient AUC scores (mean ± SD = 0.718 ± 0.034; mean ± SD = 0.628 ± 0.040, respectively) as well as in omission rates close to the predicted omission (mean difference ± SD = 0.100 ± 0.093; mean difference ± SD = 0.085 ± 0.081, respectively).

Habitat-type differentiation

In the diploid – tetraploid contact zone of *T. inodorum*, populations with different cytotype composition (diploid, tetraploid, and mixed-ploidy) showed significant differences in habitat spectrum of occupied sites in two of the three study regions ($X^2_6 = 33.9$, $P < 0.001$; Fig. 4A). In both regions, MAR and RAK, diploids and tetraploids differed in their habitat-type preferences, with tetraploid populations more commonly occurring along roadsides and at ruderal sites. The habitat

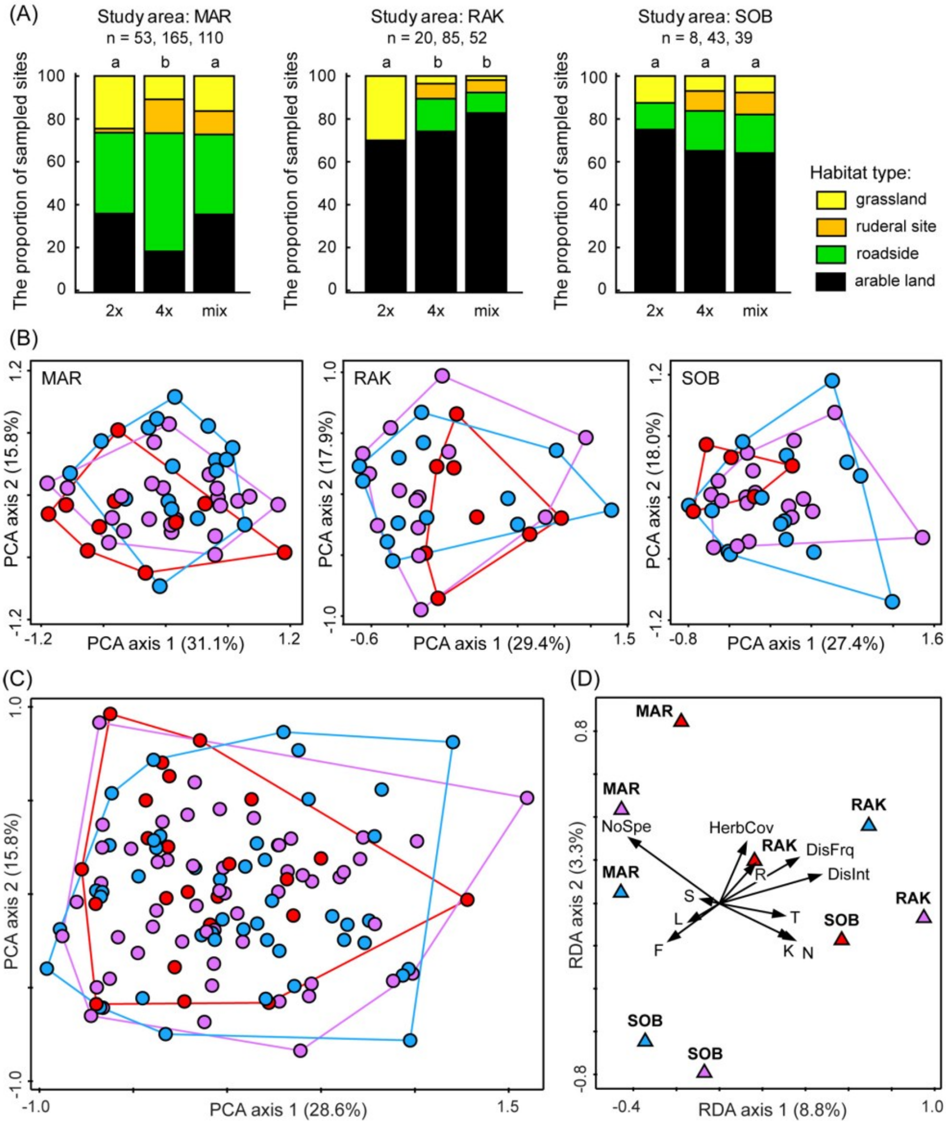

Fig. 4. Ecological-niche comparisons. (A) Relative incidence of diploid, tetraploid, and mixed-ploidy *T. inodorum* populations in the four main habitat types, shown separately for each of the three study regions. Different letters indicate significantly different groups after Bonferroni correction. (B, C) A principal component analysis of ecological variables derived from composition of vegetation in diploid (red), tetraploid (blue), and mixed-ploidy (violet) populations of the contact zone. The analysis was done separately for each study region (B) and for the complete dataset (C). (D) A redundancy analysis showing significant differences in ecological parameters of sites inhabited by a particular cytotype across all the regions. Triangles represent the centroids of diploid (red), tetraploid (blue), and mixed-ploidy (violet) populations from a particular region. Eleven variables were used for ecological-niche reconstruction in (B-D): number of species (NoSpe), relative cover of herb layer (HerbCov), index of disturbance intensity (DisInt), index of disturbance frequency (DisFrq), and Ellenberg indicator values for light (L), temperature (T), continentality (K), humidity (F), soil reaction (R), nutrients (N), and salinity (S).

spectrum of mixed-ploidy populations was similar to that of diploid populations in MAR but that of tetraploid populations in RAK. The three study regions differed in relative incidence of the four habitat types ($X^2{}_6 = 129.7$, $P < 0.001$) but not in the frequency of diploid, tetraploid, and mixed ploidy populations ($X^2{}_4 = 5.7$, $P = 0.223$). A triple interaction of ploidy composition, habitat type and study region was not significant ($X^2{}_{12} = 15.1$, $P = 0.238$).

Ecological niches of diploid, tetraploid and mixed-ploidy populations

Principal component analysis of environmental parameters (population means of ecological indicators inferred from species composition data) showed a substantial overlap of ecological niches of uniformly diploid, uniformly tetraploid, and mixed-ploidy populations. This trend was evident both in separate analyses of data from the three study regions (Fig. 4B) and in an analysis of a pooled dataset (Fig. 4C). The lack of differences among diploid, tetraploid, and mixed-ploidy populations was further indicated by non-significant role of cytotype composition in redundancy analysis (RDA; Tab. 2A). However, when the cytotype proportions in ploidy mixtures were taken into account (using percentage of diploids in populations as an explanatory variable), significant ecological differences were discovered in the region MAR and in an analysis of the complete dataset (pooled over the three regions, Tab. 2A). In the MAR region, diploid frequency in populations increased with higher disturbance frequency, disturbance intensity, herb coverage and temperature but lower salinity, humidity and light availability (Appendix S4; see Supplemental Data with the online version of this article). Interestingly, a significant interaction between the ploidy composition of populations and the region of their origin was revealed on the pooled dataset using RDA ($P = 0.0001$), suggesting that ecological niches of diploid, tetraploid, and mixed-ploidy populations differ across the three study regions (Fig. 4D). The RDA was also applied on a dataset of standard deviations of ecological parameters, capturing the variability among sub-samples in each population, which served as a proxy of microhabitat heterogeneity. Diploid, tetraploid, and mixed-ploidy populations did not differ in microhabitat heterogeneity, differences were neither found when percentage of diploids was used as an explanatory variable (Tab. 2B).

DISCUSSION

Climate as a driver of cytotype distribution patterns

The only partial overlap of climatic niches of *T. inodorum* diploids and tetraploids observed on the European scale (Fig. 2A) suggests that both cytotypes have different climatic optima in Europe. Diploid-preferred regions were characterized by lower temperature seasonality, lower mean diurnal range of temperatures, and higher mean temperature of the coldest quarter of a year, i.e., areas with oceanic climate,

Table 1. Climatic variables used in canonical discriminant analysis (CDA) and their power to differentiate climatic niches of *T. inodorum* diploids and tetraploids. Separate CDA analyses were conducted on the European and Central-European scales.

Climatic variable		European scale		Central-European scale	
Code	Description	Biplot scores[#]	P (adj.)*	Biplot scores[#]	P (adj.)*
BIO1	Annual mean temperature	-0.248	**0.0135**	–	–
BIO2	Mean diurnal range of temperatures	0.561	**0.0015**	0.637	**0.0036**
BIO3	Isothermality	-0.519	**0.0015**	0.315	0.5868
BIO4	Temperature seasonality	0.920	**0.0015**	0.495	**0.0192**
BIO5	Maximal temperature of warmest month	0.516	**0.0015**	0.486	**0.0264**
BIO6	Minimal temperature of coldest month	–	–	-0.064	1
BIO7	Temperature annual range	–	–	0.580	**0.0048**
BIO8	Mean temperature of wettest quarter	0.711	**0.0015**	–	–
BIO9	Mean temperature of driest quarter	-0.678	**0.0015**	0.126	1
BIO10	Mean temperature of warmest quarter	0.375	**0.0015**	–	–
BIO11	Mean temperature of coldest quarter	-0.697	**0.0015**	0.132	1
BIO12	Annual precipitation	-0.174	0.3255	0.316	0.5496
BIO13	Precipitation of wettest month	0.111	1	0.288	0.7884
BIO15	Precipitation seasonality	0.464	**0.0015**	-0.192	1
BIO17	Precipitation of driest quarter	-0.350	**0.0015**	–	–
BIO18	Precipitation of warmest quarter	0.368	**0.0015**	–	–
BIO19	Precipitation of coldest quarter	-0.478	**0.0015**	0.316	0.528

[#] Biplot scores of explanatory variables represent the contribution of particular climatic variables to the first canonical axis separating the cytotypes (Lepš and Šmilauer, 2014). Climatic variables with negative values of these scores are positively correlated with the diploid occurrence, variables with positive values are positively correlated with the tetraploid occurrence.

* The significance level of marginal effect of each climatic variable was adjusted using the Bonferroni correction, p-values lower than 0.05 are highlighted in bold.

Table 2. A summary of redundancy analyses (RDA) exploring the link between cytotype composition of *T. inodorum* populations and ecological parameters of inhabited sites. Eleven ecological variables were derived from species composition of each of five vegetation sub-samples taken in all populations. The RDA was applied both on a dataset of population means of ecological variables (A) and a dataset of standard deviations of variables, capturing the variability among sub-samples in each population (B).

(A) Response ecological variables	Study region	Explanatory variable	
		Ploidy composition*	Proportion of 2x[#]
population means	MAR	P = 0.0819	**P = 0.0043**[†A]
population means	RAK	P = 0.3835	P = 0.5251
population means	SOB	P =0.6686	P = 0.1603
population means	MAR + RAK + SOB	P =0.3750	**P = 0.0301**[†B]

(B) Response ecological variables	Study region	Explanatory variable	
		Ploidy composition*	Proportion of 2x[#]
variability among sub-samples	MAR	P =0.1534	P = 0.2114
variability among sub-samples	RAK	P =0.3518	P = 0.3037
variability among sub-samples	SOB	P =0.8444	P = 0.4919
variability among sub-samples	MAR + RAK + SOB	P =0.5830	P = 0.1971

Significant differences ($\alpha = 0.05$) are highlighted in bold.

*A factor with three levels (diploid, tetraploid, mixed-ploidy) characterizing the ploidy composition of populations.

[#] The percentage of diploid plants in populations.

[†] The constrained RDA axis explained [A]6.5% and [B]1.8% of overall variability.

as opposed to tetraploids that were predicted to dominate areas with continental climate (Fig. 3; in line with Kay, 1969).

If the distribution patterns of *T. inodorum* diploids and tetraploids on the European scale were indeed primarily driven by the climate, one would have expected ploidy-specific differences in biological traits (e.g. in plant physiology or life history) resulting from (pre-)adaptations of the two cytotypes to contrasting climatic optima and giving them a fitness advantage under particular environmental conditions. Polyploidy has been repeatedly associated with increased tolerance to arid environments (Levin, 2002; Treier et al., 2009; Manzaneda et al., 2012; Hao et al., 2013), and this association has been further supported by drought resistance experiments in few species (Maherali et al., 2009; Manzaneda et al., 2012). Experiments with neopolyploids have suggested that greater drought tolerance may even be a direct consequence of genome doubling (Maherali et al., 2009; Ramsey, 2011). For example, the tetraploids of *Chamerion angustifolium* have wider xylem conduits with higher hydraulic conductivity which permits them to extract more water from drying soils than diploids (Maherali et al., 2009) and niche modelling approach have shown that tetraploids indeed occupy a drier climatic niche than diploids in nature (Thompson et al., 2014).

In our study, *T. inodorum* tetraploids also tend to prefer more arid environments (characterized by e.g. lower precipitation of the driest quarter of a year and higher precipitation seasonality) than their diploid counterparts. On top of that, climatic niche modelling suggests that the mountain ranges (e.g. the Alps and Carpathians) should be predominantly occupied by diploid *T. inodorum* populations. Diploids occurring in higher altitudes than polyploids were documented in many other heteroploid model systems (e.g. Petit et al., 1997; Gauthier et al., 1998; Husband and Schemske, 1998; Sonnleitner et al., 2010) and this might reflect narrower xylem conduits of diploid plants being more resistant to freezing-induced cavitation (Davis et al., 1999), as was suggested by Thompson et al. (2014). Although it would be tempting to suppose that higher drought tolerance of tetraploids along with higher freezing tolerance of diploids could contribute to district geographic distribution of *T. inodorum* cytotypes in Europe, experimental data are needed to explain the sorting of cytotypes along climatic gradients in this species. Finally, it should be noted that the cytotypes of *T. inodorum* are likely genetically distinct (as reflected by differences in their monoploid genome sizes; Čertner et al., 2017), implying that the observed differences may not result just from genome doubling *per se* but also from post-polyploidization selection acting independently on the two cytotypes.

Apart from ecophysiological differences, contrasting large-scale cytotype distributions may also reflect mere colonization histories of cytotypes (e.g. migration from different glacial refugia) and/or their limited dispersal capabilities (Van Dijk and Bakx-Schotman, 1997; Kolář et al., 2012). In our study, however, the adaptive explanation is indirectly supported by two facts. Firstly, similar climatic factors were correlated with diploid and tetraploid occurrence on the European scale and in the zone of Central-European sympatry. Secondly, introduction of the two cytotypes to North America resulted in analogical pattern of diploids and tetraploids prevailing in oceanic and continental regions, respectively (Woo et al., 1991).

Aside from the different climatic optima of the two cytotypes on the European scale, the two cytotypes can also coexist under a wide range of climatic conditions. Climatic niche modelling has predicted suitable conditions for the coexistence of *T. inodorum* diploids and tetraploids in an extensive zone stretching from northern Italy to southern coasts of Norway and Sweden, and from central France to the Czech Republic and Baltic coast (Fig. 3). A question remains whether climatic conditions also drive the segregation within the contact zone at finer, regional, spatial scales. Climatic comparisons within the predicted contact zone (Central Europe), show only niches shared by the both cytotypes or dominated by tetraploids (Fig. 2B). The lack of diploid-dominated climatic niches at this scale, along with the fact that the easternmost parts of the study area were already predicted as unsuitable for diploids in MaxEnt modelling suggests that the diploids may be approaching their ecophysiological limits in Central Europe, whereas tetraploids are not climate-limited there. Interestingly, by comparing the MaxEnt prediction with our previous massive flow-cytometric ploidy screening of 1 209 Central-European *T. inodorum* populations (Čertner et al., 2017), diploids were not found in all the regions predicted as climatically suitable (in spite of thorough sampling in Čertner et al., 2017) and even within the occupied regions, diploids were patchily distributed. This suggests that other than climatic drivers are likely responsible for a mosaic-like structure of the cytotype contact at regional and finer scales.

Fine-scale sorting of cytotypes in the contact zone

To get better insight into the evolutionary forces governing diploid – tetraploid coexistence at smaller spatial scales, we focused at three regions of common cytotype co-occurrence (i.e. MAR, RAK, and SOB; Fig. 1) which provide replicates within the wide contact zone and thus allow for a generalization of the locally-assessed patterns. Firstly we found that *T. inodorum* populations of different cytotype composition differ in their relative occurrence across a spectrum of suitable habitat types. This difference was significant within two regions (MAR and RAK) and a similar trend was also observed in the last region (SOB) in which the lack of statistically significant differences could stem from a loss of statistical power due to lower sampling rate (Fig. 4A). Secondly, ecological indicators inferred from species composition at the occupied sites have shown a highly significant interaction between the ploidy composition of populations and the region of their origin. This even points at different ecological optima of diploid, tetraploid, and mixed-ploidy populations in each of the three regions (Fig. 4D).

Polyploidy is often associated with the origin of phenotypic novelty (Levin, 2002; Hegarty et al., 2013), and these changes may allow polyploids to occupy niches different from those of their related diploids (Lumaret et al., 1987; Sonnleitner et al., 2010). In such case, we would have expected that inherent ploidy-specific differences in plant phenotypes will lead to more-or-less consistent patterns in the ecological differentiation of cytotypes in different parts of the contact zone. However, the patterns of ecological differentiation of *T. inodorum* diploids and tetraploids observed in this study were only rarely consistent among the three study regions, despite all the regions provided similar range of ecological conditions for *T. inodorum* (Appendix S5; see Supplemental Data with the online version of this article). The only exception was proportionally higher occurrence of tetraploid populations along roadsides and at ruderal sites but lower at grasslands when compared to diploid populations (Fig. 4A). Given the lack of any consistent ecological differences in analyses of vegetation samples, the cytotypes may not have responded differentially to local ecological conditions but rather to the specific history of these habitats. Both roadside and ruderal habitats may provide only temporal suitability for *T. inodorum* and we may hypothesize that the tetraploids could be superior colonizers, could persist longer in local soil seed banks or might be able to outcompete diploids at these habitats. Despite such possible explanations need to be experimentally tested, it seems unlikely the mosaic-like structure of the cytotype contact was driven mainly by inherent ploidy-specific differences in phenotypic traits.

The surprisingly inconsistent patterns in ecological optima of di- and tetraploids among the three regions may also have an adaptive explanation. Given that inter-ploidy mating typically has a negative effect on the fitness of cytotypes (Ramsey and Schemske, 1998), selection may act upon the cytotypes co-occurring in contact zones, leading to more pronounced differences in their biological traits than observed in allopatry (Van Dijk and Bijlsma, 1994; Nuismer and Cunningham, 2005). For example, a shift in ecological niches of cytotypes may lead to lower degree of their overlap in sympatric than in allopatric populations (i.e. niche displacement in contact zone) as was repeatedly documented in diploid – polyploid contact zones (Felber-Girard et al., 1996; Ståhlberg, 2009; Sonnleitner et al., 2016). In *T. inodorum*, niche displacement acting independently in different regions of the contact zone could explain the inconsistency in ecological optima of the residing cytotypes. However, if the ecological niches of *T. inodorum* diploids and tetraploids in sympatry were indeed driven by niche displacement, we would have expected a reduced range of ecological conditions under which the cytotypes coexist within the contact zone. Contrary to that, ploidy mixtures were found to occupy almost the entire spectrum of ecological niches suitable for *T. inodorum* in each of the three regions (Fig. 4B).

Since we cannot explain the observed discrepant patterns of ecological differentiation of *T. inodorum* cytotypes in different parts of the contact zone using adaptive scenarios (i.e. ploidy-driven phenotypic differentiation, niche displacement of cytotypes in sympatry), other mechanisms must be sought. Non-adaptive scenarios for cytotype coexistence have received increased attention in the recent years. These presume that colonization history (i.e. patterns of past migration; Van Dijk and Bakx-Schotman, 1997; Kolář et al., 2012; Mráz et al., 2012), dispersal limitation (Baack, 2005), and chance events (e.g. the founder effect; Hanzl et al., 2014) may in some cases have greater impact on cytotype distribution patterns and on the conditions of cytotype coexistence than ploidy-specific differences in biological traits and/or ecological requirements. Firstly, taking into account that many sites provide only temporary favorability for annual weeds, the founder effect is expectedly playing a substantial role in the landscape dynamics of *T. inodorum* populations. Secondly, the achenes of *T. inodorum* can be effectively transferred among sites together with mud adhering to the wheels of farm vehicles or the hooves of farm animals (Kay, 1994). Once a site is colonized, a non-random transfer of migrants to/from the residing population can be expected in strongly man-influenced landscapes as farm vehicles are likely to move in between fields (meadows etc.), cattle is usually heard among close-by pastures, and traffic contributes to spread of roadside populations along communications. An interplay between initial random colonization of suitable sites by di- or tetraploids (i.e. the founder effect) and more frequent migration among sites of the same habitat type could result into distinct relative occurrences of cytotypes at particular habitat types, i.e. the same pattern as observed in *T. inodorum*. The unequal representation of di- and tetraploids at sites with specific environmental conditions could then lead to apparent "shifts" in ecological optima of cytotypes such as those we observed in analyses of vegetation samples.

The proposed combination of founder effect and non-random migration patterns not only could explain the signs of seeming ecological differentiation between *T. inodorum* cytotypes but might also contribute to a mosaic-like structure of the contact zone. Prevalence of one of the cytotypes on a local or regional scale may be further assisted by preferential transfer of *T. inodorum* achenes among sites managed by the same farm and once established, minority cytotype exclusion principle likely will be involved in maintaining the local (regional) prevalence of diploids or tetraploids (Levin, 1975; Husband, 2000). Several other plant species with ploidy heterogeneity are bound to strongly man-influenced landscapes (Meirmans et al., 1999; Halverson et al., 2008; Duchoslav et al., 2010), and we suggest the same process could be, at least partially, affecting cytotype distribution patterns in such contact zones. Nonetheless, the landscape dynamics of diploid – tetraploid contact zone in *T. inodorum* is likely even more complex due to permanent soil seed banks and presumably ongoing tetraploid expansion within the three study regions (Čertner et al., 2017).

Environmental setting for local ploidy coexistence

Within the area where climatic conditions allow co-occurrence of diploids and tetraploids, i.e. in the contact zone, no ecological factors aside from the general habitat suitability seem to constrain the formation of local ploidy mixtures. The occurrence of mixed-ploidy populations (containing both diploids and tetraploids) across nearly the entire spectrum of suitable ecological niches, as reconstructed from vegetation samples (Fig. 4C), rules out our initial hypotheses of cytotype coexistence in intermediate niches or in marginal environments, previously documented by e.g. Martin and Husband (2013), Duchoslav et al. (2010), and Sonnleitner et al. (2010), in the contact zones of *Chamerion angustifolium*, *Allium oleraceum*, and *Senecio carniolicus* s. l., respectively. Interestingly, the sites occupied by mixed-ploidy populations were subjected to comparable levels of disturbance frequency and intensity as uniform-ploidy sites, ruling out our second expectation that disturbance may have positive effect on maintaining local ploidy mixtures by preventing a majority cytotype from dominating the site (Halverson et al., 2008). Finally, cytotype coexistence was neither favored at sites displaying higher degree of microhabitat heterogeneity (i.e. variability among sub-samples in each population), although such small-scale heterogeneity may contribute to cytotype coexistence (Sonnleitner et al., 2010). In sum, the lack of environmental constraints is most likely responsible for the frequent incidence of mixed-ploidy populations in the diploid – tetraploid contact zone of *T. inodorum* (33-43% in the three studied regions; Čertner et al., 2017). Rather than local ecological conditions, the proximity of diploid and tetraploid source populations along with local patterns of migration, i.e. non-adaptive processes, may determine where the *T. inodorum* cytotypes meet at a local scale.

Conclusions

Our investigation of environmental factors driving the occurrence of *T. inodorum* cytotypes in Europe was conducted at several spatial scales, ranging from population to continental level. Large-scale analyses of climatic niches have revealed that diploids prefer areas with oceanic climate, whereas tetraploids favor areas with the contrasting continental climate, and we thus expect climate being an important factor determining both the position and breath of the contact zone. However, within the contact zone, at a regional scale, we did not observe any constant differences in ecological preferences of the co-existing cytotypes, in spite of our thorough search conducted independently in three regions. As the pattern could neither be explained by ecological niche displacement in sympatry, we suggest a non-adaptive scenario that is based on stochastic colonization of newly available suitable sites by di- or tetraploids, anthropogenic activities causing non-random migration patterns in strongly man-influenced landscapes, and, finally, on minority cytotype disadvantage maintaining the established local prevalence of one of the cytotypes. Within the contact zone, we have not identified any ecological constraints to cytotype coexistence, which corresponds with the observed ubiquity of mixed-ploidy populations in *T. inodorum*.

The present study demonstrates that different environmental forces and biological processes may shape cytotype distribution patterns at different spatial scales. In general, studies of environmental drivers of cytotype coexistence only rarely operate at more than one spatial scale, and since contrasting patterns may be found at various scales, the scale selection may substantially affect conclusions reached (Hanzl et al., 2014; Kirchheimer et al., 2016). Another possible pitfall is assessing the actual contribution of environmental heterogeneity to facilitating cytotype coexistence. Non-random geographical distribution of cytotypes may frequently lead to significant associations between probability of a cytotype occurrence and values of some environmental variable, albeit not always is there a causal link. In this study, we attempted to overcome this problem by looking for similar trends across different spatial scales and in multiple geographical regions within a particular spatial scale. However, an experimental comparison of ecophysiology of *T. inodorum* cytotypes is still needed to confirm or reject the suggested causes for climatically-driven differentiation.

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LITERATURE CITED

Baack, E.J. 2005. To succeed globally, disperse locally: effects of local pollen and seed dispersal on tetraploid establishment. *Heredity* 94: 538–546.

Baack, E.J., and M.L. Stanton. 2005. Ecological factors influencing tetraploid speciation in snow buttercups (*Ranunculus adoneus*): niche differentiation and tetraploid establishment. *Evolution* 59: 1936–1944.

Barringer, B.C. 2007. Polyploidy and self-fertilization in flowering plants. *American Journal of Botany* 94: 1527–1533.

Buggs, R.J.A., and J.R. Pannell. 2007. Ecological differentiation and diploid superiority across a moving ploidy contact zone. *Evolution* 61: 125–140.

Coyne, J.A., and H.A. Orr. 2004. Speciation. Sinauer Associates, Sunderland.

Čertner, M., E. Fenclová, P. Kúr, F. Kolář, P. Koutecký, A. Krahulcová, and J. Suda. 2017. Evolutionary dynamics of mixed-ploidy populations in an annual herb: dispersal, local persistence and recurrent origins of polyploids. *Annals of Botany* 120: 303–315.

Davis, S.D., J.S. Sperry, and U.G. Hacke. 1999. The relationship between xylem conduit diameter and cavitation caused by freezing. *American Journal of Botany* 86: 1367–1372.

Van Dijk, P., and T. Bakx-Schotman. 1997. Chloroplast DNA phylogeography and cytotype geography in autopolyploid *Plantago media*. *Molecular Ecology* 6: 345–352.

Van Dijk, P., and R. Bijlsma. 1994. Simulations of flowering time displacement between two cytotypes that form inviable hybrids. *Heredity* 72: 522–535.

Duchoslav, M., L. Šafářová, and F. Krahulec. 2010. Complex distribution patterns, ecology and coexistence of ploidy levels of *Allium oleraceum* (Alliaceae) in the Czech Republic. *Annals of Botany* 105: 719–735.

Ehrendorfer, F. 1980. Polyploidy and distribution. *In* W. H. Lewis [ed.], Polyploidy: biological relevance, 45–60. Plenum Press, New York.

Ellenberg, H., H.E. Weber, R. Düll, V. Wirth, and W. Werner. 2001. Zeigerwerte von Pflanzen in Mitteleuropa. 3rd ed. Goltze, Göttlingen.

Felber-Girard, M., F. Felber, and A. Buttler. 1996. Habitat differentiation in a narrow hybrid zone between diploid and tetraploid *Anthoxanthum alpinum*. *New Phytologist* 133: 531–540.

Fielding, A., and J. Bell. 1997. A review of methods for the assessment of prediction errors in conservation presence/absence models. *Environmental Conservation* 24: 38–49.

Flagel, L.E., and J.F. Wendel. 2009. Gene duplication and evolutionary novelty in plants. *New Phytologist* 183: 557–564.

Fowler, N.L., and D.A. Levin. 1984. Ecological constraints on the establishment of a novel polyploid in competition with its diploid progenitor. *The American Naturalist* 124: 703–711.

Gauthier, P., R. Lumaret, and A. Bédécarrats. 1998. Genetic variation and gene flow in Alpine diploid and tetraploid populations of *Lotus* (*L. alpinus* (D.C.) Schleicher/*L. corniculatus* L.). II. Insights from RFLP of chloroplast DNA. *Heredity* 80: 694–701.

Glennon, K.L., M.E. Ritchie, and K. a. Segraves. 2014. Evidence for shared broad-scale climatic niches of diploid and polyploid plants. *Ecology Letters* 17: 574–582.

Halverson, K., S.B. Heard, J.D. Nason, and J.O. Stireman. 2008. Origins, distribution, and local co-occurrence of polyploid cytotypes in *Solidago altissima* (Asteraceae). *American Journal of Botany* 95: 50–58.

Hanzl, M., F. Kolář, D. Nováková, and J. Suda. 2014. Nonadaptive processes governing early stages of polyploid evolution: Insights from a primary contact zone of relict serpentine *Knautia arvensis* (Caprifoliaceae). *American Journal of Botany* 101: 935–945.

Hao, G.Y., M.E. Lucero, S.C. Sanderson, E.H. Zacharias, and N.M. Holbrook. 2013. Polyploidy enhances the occupation of heterogeneous environments through hydraulic related trade-offs in *Atriplex canescens* (Chenopodiaceae). *New Phytologist* 197: 970–978.

Hegarty, M., J. Coate, S. Sherman-Broyles, R. Abbott, S. Hiscock, and J. Doyle. 2013. Lessons from natural and artificial polyploids in higher plants. *Cytogenetic and Genome Research* 140: 204–225.

Herben, T., M. Chytrý, and J. Klimešová. 2016. A quest for species-level indicator values for disturbance. *Journal of Vegetation Science* 27: 628–636.

Hijmans, R.J., S.E. Cameron, J.L. Parra, G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–1978.

Husband, B.C. 2000. Constraints on polyploid evolution: A test of the minority cytotype exclusion principle. *Proceedings of the Royal Society B-Biological Sciences* 267: 217–223.

Husband, B.C., and D.W. Schemske. 1998. Cytotype distribution at a diploid-tetraploid contact zone in *Chamerion* (*Epilobium*) *angustifolium* (Onagraceae). *American Journal of Botany* 85: 1688–1694.

Kay, Q.O.N. 1976. 60. *Matricaria* L. *In* T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, and D. A. Webb [eds.], Flora Europaea, 165–167. Cambridge University Press, Cambridge.

Kay, Q.O.N. 1969. The origin and distribution of diploid and tetraploid *Tripleurospermum inodorum* (L.) Schultz Bip. *Watsonia* 7: 130–141.

Kay, Q.O.N. 1994. *Tripleurospermum inodorum* (L.) Schultz Bip. *Journal of Ecology* 82: 681–697.

Kirchheimer, B., C.C.F. Schinkel, A.S. Dellinger, S. Klatt, D. Moser, M. Winkler, J. Lenoir, et al. 2016. A matter of scale: apparent niche differentiation of diploid and tetraploid plants may depend on extent and grain of analysis. *Journal of Biogeography* 43: 716–726.

Kolář, F., T. Fér, M. Štech, P. Trávníček, E. Dušková, P. Schönswetter, and J. Suda. 2012. Bringing together evolution on serpentine and polyploidy: Spatiotemporal history of the diploid-tetraploid complex of *Knautia arvensis* (Dipsacaceae). *PLoS ONE* 7: e39988.

Kubát, K. 2004. 33. *Tripleurospermum* Schultz Bip. - heřmánkovec. *In* B. Slavík, and J. Štěpánková [eds.], Květena České republiky, 248–250. Academia, Praha.

Lankosz-Mróz, M. 1976. Karyological investigations on *Tripleurospermum maritimum* (L.) Koch ssp. *inodorum* (L.) ex Vaarama from Poland. *Acta Biologica Cracoviensia (Series Botanica)* 19: 93–105.

Laport, R.G., L. Hatem, R.L. Minckley, and J. Ramsey. 2013. Ecological niche modeling implicates climatic adaptation, competitive exclusion, and niche conservatism among *Larrea tridentata* cytotypes in North American deserts. *The Journal of the Torrey Botanical Society* 140: 349–363.

Lepš, J., and P. Šmilauer. 2014. Multivariate Analysis of Ecological Data using CANOCO 5. 2nd ed. Cambridge University Press, Cambridge.

Levin, D.A. 1975. Minority cytotype exclusion in local plant populations. *Taxon* 24: 35–43.

Levin, D.A. 2002. The role of chromosomal change in plant evolution. Oxford University Press, Oxford.

Lumaret, R., J.L. Guillerm, J. Delay, A. Ait Lhaj Loutfi, J. Izco, and M. Jay. 1987. Polyploidy and habitat differentiation in *Dactylis glomerata* L. from Galicia (Spain). *Oecologia* 73: 436–446.

Maherali, H., A.E. Walden, and B.C. Husband. 2009. Genome duplication and the evolution of physiological responses to water stress. *New Phytologist* 184: 721–731.

Manzaneda, A.J., P.J. Rey, J.M. Bastida, C. Weiss-Lehman, E. Raskin, and T. Mitchell-Olds. 2012. Environmental aridity is associated with cytotype segregation and polyploidy occurrence in *Brachypodium distachyon* (Poaceae). *The New Phytologist* 193: 797–805.

Martin, S.L., and B.C. Husband. 2013. Adaptation of diploid and tetraploid *Chamerion angustifolium* to elevation but not local environment. *Evolution* 67: 1780–1791.

Meirmans, P.G., F.G. Calame, F. Bretagnolle, F. Felber, and J.C.M. den Nijs. 1999. Anthropogenic disturbance and habitat differentiation between sexual diploid and apomictic triploid *Taraxacum* sect. *Ruderalia*. *Folia Geobotanica* 34: 451–469.

Mráz, P., S. Španiel, A. Keller, G. Bowmann, A. Farkas, B. Šingliarová, R.P. Rohr, et al. 2012. Anthropogenic disturbance as a driver of microspatial and microhabitat segregation of cytotypes of *Centaurea stoebe* and cytotype interactions in secondary contact zones. *Annals of Botany* 110: 615–627.

Nuismer, S.L., and B.M. Cunningham. 2005. Selection for phenotypic divergence between diploid and autotetraploid *Heuchera grossulariifolia*. *Evolution* 59: 1928–1935.

Petit, C., F. Bretagnolle, and F. Felber. 1999. Evolutionary consequences of diploid-polyploid hybrid zones in wild species. *Trends in Ecology and Evolution* 14: 306–311.

Petit, C., P. Lesbros, X. Ge, and J.D. Thompson. 1997. Variation in flowering phenology and selfing rate across a contact zone between diploid and tetraploid *Arrhenatherum elatius* (Poaceae). *Heredity* 79: 31–40.

Phillips, S.J., and M. Dudík. 2008. Modeling of species distribution with Maxent: New extensions and a comprehensive evalutation. *Ecography* 31: 161–175.

Phillips, S.J., M. Dudík, J. Elith, C.H. Graham, A. Lehmann, J. Leathwick, and S. Ferrier. 2009. Sample selection bias and presence-only distribution models : Implications for background and pseudo-absence data. *Ecological Applications* 19: 181–197.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramsey, J. 2011. Polyploidy and ecological adaptation in wild yarrow. *Proceedings of the National Academy of Sciences of the United States of America* 108: 7096–7101.

Ramsey, J., and T.S. Ramsey. 2014. Ecological studies of polyploidy in the 100 years following its discovery. *Philosophical Transactions of the Royal Society B: Biological Sciences* 369: 20130352.

Ramsey, J., and D.W. Schemske. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* 29: 467–501.

Rodríguez, D.J. 1996. A model for the establishment of polyploidy in plants. *The American Naturalist* 147: 33–46.

Segraves, K.A., and J.N. Thompson. 1999. Plant polyploidy and pollination: Floral traits and insect visits to diploid and tetraploid *Heuchera grossulariifolia*. *Evolution* 53: 1114–1127.

Soltis, D.E., V.A. Albert, J. Leebens-Mack, C.D. Bell, A.H. Paterson, C. Zheng, D. Sankoff, et al. 2009. Polyploidy and angiosperm diversification. *American Journal of Botany* 96: 336–348.

Sonnleitner, M., R. Flatscher, P. Escobar García, J. Rauchová, J. Suda, G.M. Schneeweiss, K. Hülber, and P. Schönswetter. 2010. Distribution and habitat segregation on different spatial scales among diploid, tetraploid and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps. *Annals of Botany* 106: 967–977.

Sonnleitner, M., K. Hülber, R. Flatscher, P.E. García, M. Winkler, J. Suda, P. Schönswetter, and G.M. Schneeweiss. 2016. Ecological differentiation of diploid and polyploid cytotypes of *Senecio carniolicus* sensu lato (Asteraceae) is stronger in areas of sympatry. *Annals of Botany* 117: 269–276.

Ståhlberg, D. 2009. Habitat differentiation, hybridization and gene flow patterns in mixed populations of diploid and autotetraploid *Dactylorhiza maculata* s.l. (Orchidaceae). *Evolutionary Ecology* 23: 295–328.

Thompson, K.A., B.C. Husband, and H. Maherali. 2014. Climatic niche differences between diploid and tetraploid cytotypes of *Chamerion angustifolium* (Onagraceae). *American Journal of Botany* 101: 1868–1875.

Trávníček, P., Z. Dočkalová, R. Rosenbaumová, B. Kubátová, Z. Szelag, and J. Chrtek. 2011. Bridging global and microregional scales: Ploidy distribution in *Pilosella echioides* (Asteraceae) in central Europe. *Annals of Botany* 107: 443–454.

Treier, U.A., O. Broennimann, S. Normand, A. Guisan, U. Schaffner, T. Steinger, and H. Müller-Schärer. 2009. Shift in cytotype frequency and niche space in the invasive plant *Centaurea maculosa*. *Ecology* 90: 1366–1377.

Wendel, J.F., S.A. Jackson, B.C. Meyers, and R.A. Wing. 2016. Evolution of plant genome architecture. *Genome Biology* 17: 37.

Woo, S.L., A.G. Thomas, D.P. Peschken, G.G. Bowes, D.W. Douglas, V.L. Harms, and A.S. McClay. 1991. The biology of Canadian weeds. 99. *Matricaria perforata* Mérat (Asteraceae). *Canadian Journal of Plant Science* 71: 1101–1119.

Wood, T.E., N. Takebayashi, M.S. Barker, I. Mayrose, P.B. Greenspoon, and L.H. Rieseberg. 2009. The frequency of polyploid speciation in vascular plants. *Proceedings of the National Academy of Sciences of the United States of America* 106: 13875–13879.

Appendix S1. List of 44 *Tripleurospermum inodorum* populations sampled for ploidy composition in our study.

Locality	Coordinates (WGS-84)	Date	Sample size*	Ploidy level†
Denmark, Møn island, Magleby, on a field 0.5 km E of the town	54°59'07.5"N, 12°29'50.8"E	10. 9. 2014	13	2x
France, Besançon, on a ruderal site at a river bank in the town	47°13'53.8"N, 06°02'16.8"E	22. 8. 2014	5	2x
France, Les Chazes, on a ruderal site at the NW edge of the town	45°04'17.3"N, 02°42'51.1"E	20. 8. 2014	2	2x
Netherlands, Amsterdam, on a ruderal site by a bridge under the highway A10	52°23'29.3"N, 04°50'37.6"E	7. 12. 2013	5	2x
Poland, Komnino, on a roadside S of the settlement	54°36'22.6"N, 17°07'21.8"E	20. 9. 2014	7	2x
Poland, Koszalin, on a ruderal site by a petrol station at the NW edge of the town	54°12'34.9"N, 16°09'14.4"E	20. 9. 2014	1	2x
Poland, Lubiatowo, on a ruderal site 0.5 km N of the town	54°48'17.0"N, 17°51'26.1"E	21. 9. 2014	8	2x
United Kingdom, Glenfarg, on a roadside 3.5 km E of the town	56°16'39.2"N, 03°20'40.0"W	18. 10. 2011	5	2x
Slovenia, Srednja vas v Bohinju, on a roadside at Rudno polje parking	46°20'47.0"N, 13°55'27.3"E	11. 8. 2014	11	2x (1), 4x (10)
United Kingdom, Taunton, on a roadside by a bridge under the highway M5	51°01'03.7"N, 03°03'43.3"W	12. 9. 2014	7	2x (6), 3x (1)
Austria, Treffen am Ossiacher See, on a field 1 km SE of the town	46°39'28.3"N, 13°52'03.4"E	25. 6. 2014	8	4x
Denmark, Helsingør, on a ruderal site N of Kronborg castle	56°02'23.8"N, 12°37'21.9"E	24. 7. 2013	3	4x
Denmark, Hillerød, on a ruderal site in park N of Frederiksborg Slott	55°56'10.1"N, 12°18'01.3"E	24. 7. 2013	4	4x
Finland, Lammi, on a field 6 km N of the town	61°08'13.2"N, 25°00'07.0"E	19. 8. 2012	10	4x
France, Villar-d'Arêne, on a parking lot 4 km SE of the town (road to Briançon)	45°01'58.1"N, 06°23'03.1"E	24. 8. 2014	6	4x
Hungary, Akasztó, on a roadside 4 km NW of the town (road to Dunatetétlen)	46°43'34.6"N, 19°10'14.1"E	29. 8. 2015	4	4x
Hungary, Dunaföldvár, on a ruderal site 1 km E of the town	46°48'51.4"N, 18°56'20.8"E	29. 8. 2015	1	4x
Norway, Asker, on a disturbed grassland 0.5 km NE of Vettre	59°49'49.9"N, 10°29'10.4"E	11. 10. 2014	2	4x
Norway, Drammen, on a roadside by a petrol station at the E edge of the town	59°44'58.4"N, 10°17'20.9"E	13. 8. 2013	5	4x
Norway, Oslo-Haslum, on a field 0.5km NW of Dælivannet lake	59°55'30.9"N, 10°32'17.3"E	3. 10. 2015	10	4x
Norway, Sørbråten, on a ruderal site on a farm 1 km W of the town	60°01'30.1"N, 10°47'06.7"E	27. 9. 2015	6	4x
Norway, Varberg, on a roadside by a petrol station 4.5 km N of the town	57°10'05.2"N, 12°16'35.3"E	13. 8. 2013	5	4x

Poland, Boręty, on a field drive 1 km NW of the town	54°08'33.9"N, 18°50'09.5"E	21. 9. 2014	1	4x
Poland, Czatkowy, on a field 1 km E of the town	54°07'50.2"N, 18°49'57.1"E	21. 9. 2014	10	4x
Poland, Czatkowy, on a field drive 1.5 km SE of the town	54°07'17.2"N, 18°49'57.0"E	21. 9. 2014	5	4x
Poland, Derczewo, on a roadside at a pull off 2 km NW of the town	53°03'07.5"N, 14°52'01.8"E	30. 7. 2013	5	4x
Poland, Gniazdowo, on a ruderal site on a bank of the Wisła river	54°12'18.5"N, 18°55'08.4"E	21. 9. 2014	1	4x
Poland, Gryfino, on a roadside by a bridge over the Odra Zachodnia river	53°15'16.4"N, 14°26'34.5"E	19. 9. 2014	3	4x
Poland, Jarszewo, on a field 1.5 km SE of the town	53°55'04.7"N, 14°48'18.5"E	19. 9. 2014	10	4x
Poland, Ostaszewo, on a field 1 km SW of the town	54°12'10.8"N, 18°56'40.9"E	21. 9. 2014	9	4x
Poland, Widuchowa, on a roadside at the E edge of the town	53°07'36.4"N, 14°23'52.7"E	19. 9. 2014	2	4x
Romania, Agnita, on a pasture at the E edge of the town	45°58'54.2"N, 24°39'08.4"E	19. 7. 2014	3	4x
Romania, Bădeni, on a roadside in the town	46°13'02.1"N, 25°20'35.5"E	27. 6. 2012	3	4x
Romania, Borşa, on a ruderal site at the S edge of the town	47°37'51.4"N, 24°39'41.1"E	16. 7. 2014	3	4x
Romania, Iaşi, on a field N of Valea Ursului	47°08'32.3"N, 27°29'43.9"E	30. 6. 2012	5	4x
Romania, Măneciu-Ungureni, on a roadside at the NW edge of the town	45°19'41.3"N, 25°58'45.6"E	30. 6. 2012	1	4x
Romania, Praid, on a roadside in the town	46°32'31.7"N, 25°06'40.2"E	24. 6. 2012	4	4x
Romania, Sânpaul, on a ruderalised salt marsh 1 km E of the town	46°10'60.0"N, 25°23'42.0"E	27. 6. 2012	6	4x
Romania, Târgu Mureş, on a ruderal site near a parking lot SE of the town	46°31'52.2"N, 24°35'50.5"E	30. 6. 2012	5	4x
Romania, Tigveni, on a pasture 1.5 km E of the town	45°08'27.8"N, 24°35'30.4"E	20. 7. 2014	2	4x
Romania, Traian, on a roadside 3 km W of the town	46°36'49.7"N, 26°59'11.6"E	30. 6. 2012	3	4x
Romania, Vadu Crişului, on a roadside 4 km SW of the town	46°56'59.0"N, 22°29'22.1"E	22. 6. 2012	5	4x
Sweden, Öland island, Kalkstad, on a ruderal site in the town	56°36'33.3"N, 16°31'26.9"E	31. 7. 2013	5	4x
Sweden, Tanumshede, on a roadside by a petrol station E of the town	58°43'14.8"N, 11°21'00.4"E	10. 9. 2014	4	4x

*Number of individuals analyzed for ploidy level.

[†]Cytotypes found among the samples. In case of mixed-ploidy populations, number of individuals belonging to particular cytotype is stated in brackets.

Appendix S2. Sampling information for 123 diploid, tetraploid and mixed-ploidy populations of *T. inodorum* located in three regions witin the diploid – tetraploid contact zone. In these populations, species composition of vegetation was assessed and used for reconstruction of ecological niches.

Pop. code	**Region**	**Latitude**	**Longitude**	**Habitat**	**Date**	**Ploidy**	**Proportion of 2x** [%]
1	MAR	49.860978	12.737641	field	3.7.2014	4x	0
2	MAR	49.858208	12.742690	fallow	3.7.2014	4x	0
3	MAR	49.854753	12.771420	field	3.7.2014	4x	0
4	MAR	49.861328	12.769053	field	3.7.2014	2x + 4x	10
5	MAR	49.865663	12.761766	field	3.7.2014	2x	100
6	MAR	49.870619	12.766078	field	3.7.2014	2x + 4x	30
7	MAR	49.873741	12.748821	field	5.7.2014	2x	100
8	MAR	49.884623	12.759678	field	5.7.2014	2x	100
9	MAR	49.890287	12.764705	field	5.7.2014	2x + 4x	90
10	MAR	49.888831	12.771751	fallow	5.7.2014	2x	100
11	MAR	49.892565	12.762017	roadside	5.7.2014	2x + 4x	90
12	MAR	49.893186	12.744278	field drive	5.7.2014	2x + 4x	50
13	MAR	49.886174	12.727161	field	5.7.2014	2x + 4x	80
14	MAR	49.948939	12.695408	ruderal site	14.7.2014	4x	0
15	MAR	49.943165	12.672539	ruderal site	14.7.2014	2x + 4x	90
16	MAR	49.941594	12.646605	roadside	14.7.2014	2x + 4x	20
17	MAR	49.976487	12.765556	ruderal site	15.7.2014	4x	0
18	MAR	49.992231	12.805101	roadside	15.7.2014	4x	0
19	MAR	49.992811	12.842439	field	15.7.2014	2x + 4x	90
20	MAR	49.963005	12.869641	ruderal site	15.7.2014	2x + 4x	20
21	MAR	49.964938	12.857613	field	15.7.2014	4x	0
22	MAR	49.954261	12.837417	meadow	15.7.2014	2x	100
23	MAR	49.988868	12.583368	pasture	16.7.2014	2x + 4x	80
24	MAR	49.986197	12.576394	fallow	16.7.2014	2x + 4x	50
25	MAR	49.981822	12.559364	meadow	16.7.2014	2x + 4x	10
26	MAR	49.980249	12.594759	pasture	16.7.2014	4x	0
27	MAR	49.953528	12.613165	fallow	16.7.2014	2x	100
28	MAR	49.943874	12.629415	field	16.7.2014	2x + 4x	40
29	MAR	50.081111	12.762399	meadow	17.7.2014	4x	0
30	MAR	49.921245	12.676741	field drive	31.7.2014	2x + 4x	60
31	MAR	49.901095	12.668751	field	31.7.2014	4x	0
32	MAR	49.877096	12.709500	field	31.7.2014	2x + 4x	60
33	MAR	49.874968	12.724994	fallow	31.7.2014	2x + 4x	10
34	MAR	49.933425	12.751918	fallow	1.8.2014	4x	0
35	MAR	49.924262	12.764290	pasture	1.8.2014	2x + 4x	50
36	MAR	49.883149	12.726568	railway	1.8.2014	4x	0
37	MAR	49.863704	12.697220	pond bottom	1.8.2014	2x + 4x	10
38	MAR	49.866303	12.663273	fallow	1.8.2014	2x + 4x	70
39	MAR	49.870994	12.656551	roadside	1.8.2014	4x	0
40	MAR	49.886711	12.629850	field	1.8.2014	2x	100
41	MAR	49.928379	12.613375	field	1.8.2014	2x	100
42	MAR	50.062605	12.741134	meadow	2.8.2014	2x	100
43	MAR	50.032414	12.755368	forest drive	2.8.2014	2x + 4x	20
44	MAR	50.035941	12.784803	field drive	2.8.2014	4x	0
45	MAR	50.029063	12.802114	roadside	2.8.2014	4x	0
46	MAR	50.010860	12.812174	roadside	2.8.2014	4x	0
47	MAR	49.996900	12.797257	forest drive	2.8.2014	2x + 4x	80
48	MAR	49.940061	12.848956	pasture	3.8.2014	2x	100
49	MAR	49.914117	12.871112	roadside	3.8.2014	2x + 4x	30
50	MAR	49.903849	12.828686	ruderal site	3.8.2014	4x	0
51	RAK	50.251720	13.746370	field	23.7.2014	2x	100

52	RAK	50.224480	13.732280	field	23.7.2014	2x + 4x	30
53	RAK	50.222830	13.701420	field	23.7.2014	2x	100
54	RAK	50.221020	13.588520	fallow	29.7.2014	2x + 4x	60
55	RAK	50.167900	13.647430	field	29.7.2014	2x + 4x	70
56	RAK	50.132810	13.635450	field	29.7.2014	2x + 4x	10
57	RAK	50.070270	13.590660	field	29.7.2014	2x	100
58	RAK	50.045200	13.736300	field	29.7.2014	2x + 4x	10
59	RAK	50.151410	13.864780	field	29.7.2014	4x	0
60	RAK	50.117520	13.777310	field	30.7.2014	2x	100
61	RAK	50.105160	13.666370	field	31.7.2014	4x	0
62	RAK	50.108090	13.600460	field	31.7.2014	2x + 4x	40
63	RAK	50.108710	13.577280	field	31.7.2014	2x + 4x	40
64	RAK	50.108070	13.580900	field	31.7.2014	2x + 4x	70
65	RAK	50.127610	13.550560	field	31.7.2014	4x	0
66	RAK	50.184010	13.744290	field	1.8.2014	4x	0
67	RAK	50.199410	13.712980	field	1.8.2014	4x	0
68	RAK	50.214390	13.711200	field	1.8.2014	4x	0
69	RAK	50.224230	13.728380	fallow	1.8.2014	2x + 4x	30
70	RAK	50.122420	13.785050	pasture	11.8.2014	2x	100
71	RAK	50.118690	13.733720	field	11.8.2014	2x	100
72	RAK	50.128170	13.727170	field	11.8.2014	2x + 4x	80
73	RAK	50.158620	13.744260	fallow	11.8.2014	4x	0
74	RAK	50.161580	13.743780	field	11.8.2014	2x + 4x	80
75	RAK	50.203260	13.746560	field drive	11.8.2014	2x	100
76	RAK	50.225390	13.723500	field drive	11.8.2014	2x + 4x	20
77	RAK	50.225090	13.716900	field	12.8.2014	2x + 4x	10
78	RAK	50.279290	13.682380	field drive	12.8.2014	4x	0
79	RAK	50.294170	13.634530	field	12.8.2014	4x	0
80	RAK	50.234250	13.588520	field	12.8.2014	4x	0
81	RAK	50.195060	13.663830	field	12.8.2014	2x + 4x	10
82	RAK	50.174180	13.697020	hop field	12.8.2014	4x	0
83	RAK	50.193070	13.797790	field	13.8.2014	4x	0
84	RAK	50.266890	13.708500	pasture	13.8.2014	2x	100
85	RAK	50.282700	13.625020	meadow	13.8.2014	4x	0
86	SOB	49.307295	14.950939	field	10.7.2014	4x	0
87	SOB	49.306594	14.952745	roadside	10.7.2014	4x	0
88	SOB	49.323360	14.839928	roadside	10.7.2014	2x + 4x	30
89	SOB	49.322496	14.839102	ruderal site	10.7.2014	2x + 4x	60
90	SOB	49.324888	14.841341	field	10.7.2014	4x	0
91	SOB	49.341770	14.833280	field	10.7.2014	2x + 4x	70
92	SOB	49.345284	14.834572	ruderal site	10.7.2014	2x + 4x	10
93	SOB	49.374846	14.842537	meadow	10.7.2014	4x	0
94	SOB	49.341305	14.794759	roadside	10.7.2014	2x	100
95	SOB	49.328988	14.786909	field	10.7.2014	2x + 4x	90
96	SOB	49.311833	14.789139	field	10.7.2014	2x + 4x	30
97	SOB	49.322168	14.737093	roadside	10.7.2014	2x + 4x	30
98	SOB	49.288229	14.747542	ruderal site	10.7.2014	4x	0
99	SOB	49.259941	14.781179	pond bottom	10.7.2014	4x	0
100	SOB	49.235501	14.953738	roadside	11.7.2014	4x	0
101	SOB	49.232876	14.955413	field	11.7.2014	2x + 4x	10
102	SOB	49.195451	14.957816	roadside	11.7.2014	2x + 4x	10
103	SOB	49.208946	14.943801	field	11.7.2014	2x + 4x	90
104	SOB	49.181805	14.888313	field	11.7.2014	4x	0
105	SOB	49.228787	14.881623	fallow	11.7.2014	2x + 4x	20
106	SOB	49.236600	14.836703	field	11.7.2014	2x + 4x	40
107	SOB	49.241153	14.761095	field drive	11.7.2014	4x	0
108	SOB	49.261378	14.785927	pond bottom	11.7.2014	4x	0
109	SOB	49.279771	14.898408	fallow	11.7.2014	2x	100

110	SOB	49.259463	14.893787	field	11.7.2014	2x + 4x	70
111	SOB	49.266635	14.893630	fallow	12.7.2014	4x	0
112	SOB	49.293451	14.850478	field	12.7.2014	2x + 4x	50
113	SOB	49.280439	14.866669	field	12.7.2014	2x	100
114	SOB	49.258980	14.806494	field	5.8.2014	4x	0
115	SOB	49.273500	14.868028	field	5.8.2014	2x	100
116	SOB	49.269944	14.877278	field	5.8.2014	2x + 4x	90
117	SOB	49.261750	14.881250	field	5.8.2014	2x	100
118	SOB	49.256889	14.884139	pond bottom	5.8.2014	2x + 4x	20
119	SOB	49.376833	14.770278	pond bottom	5.8.2014	4x	0
120	SOB	49.243058	14.817382	meadow	5.8.2014	2x + 4x	20
121	SOB	49.236722	14.836833	field	5.8.2014	4x	0
122	SOB	49.255897	14.914889	ruderal site	5.8.2014	4x	0
123	SOB	49.252278	14.909528	pond bottom	5.8.2014	2x + 4x	60

Appendix S3. List of accompanying vascular plant species recorded in 123 diploid, tetraploid and mixed-ploidy populations of *T. inodorum*. Numeric population codes correspond to Appendix S2, five replicate vegetation samples (A-E) were assessed in each population.

1A (herb cover 45%): *Brassica napus, Daucus carota, Elytrigia repens, Epilobium tetragonum, Galium aparine, Geranium dissectum, Hordeum vulgare, Myosotis arvensis, Veronica persica, Vicia tetrasperma*
1B (herb cover 55%): *Apera spica-venti, Elytrigia repens, Epilobium tetragonum, Festuca rubra, Geranium dissectum, Hordeum vulgare, Lolium perenne, Myosotis arvensis, Veronica arvensis, V. persica, Vicia tetrasperma*
1C (herb cover 70%): *Elytrigia repens, Epilobium tetragonum, Festuca rubra, Galium spurium, Hypericum perforatum, Lolium multiflorum, Myosotis arvensis, Poa trivialis, Tanacetum vulgare, Vicia tetrasperma, Vulpia myuros*
1D (herb cover 50%): *Cirsium arvense, Fallopia convolvulus, Festuca rubra, Galium aparine, Matricaria recutita, Medicago lupulina, Pastinaca sativa, Polygonum aviculare, Veronica arvensis*
1E (herb cover 90%): *Brassica napus, Galium aparine, Myosotis arvensis, Veronica arvensis, V. persica, Vicia hirsuta, V. tetrasperma*
2A (herb cover 45%): *Agrostis capillaris, Bromus hordeaceus, Cirsium arvense, Elytrigia repens, Glechoma hederacea, Lathyrus pratensis, Plantago major, Poa trivialis, Taraxacum* sect. *Ruderalia, Veronica arvensis*
2B (herb cover 85%): *Cirsium arvense, Elytrigia repens, Lathyrus pratensis, Poa trivialis, Taraxacum* sect. *Ruderalia*
2C (herb cover 45%): *Bromus hordeaceus, Carex ovalis, Cirsium arvense, Elytrigia repens, Epilobium tetragonum, Glechoma hederacea, Lathyrus pratensis, Pilosella* sp., *Poa trivialis, Taraxacum* sect. *Ruderalia, Veronica arvensis*
2D (herb cover 65%): *Elytrigia repens, Epilobium tetragonum, Glechoma hederacea, Taraxacum sect. Ruderalia*
2E (herb cover 50%): *Bromus hordeaceus, Cirsium arvense, Elytrigia repens, Euphorbia helioscopia, Glechoma hederacea, Taraxacum* sect. *Ruderalia*
3A (herb cover 60%): *Anthemis arvensis, Apera spica-venti, Aphanes arvensis, Arabidopsis thaliana, Centaurea cyanus, Cirsium arvense, Matricaria recutita, Papaver rhoeas, Poa pratensis, Taraxacum* sect. *Ruderalia, Triticum aestivum, Veronica arvensis, Vicia hirsuta, Viola arvensis*
3B (herb cover 80%): *Anthemis arvensis, Apera spica-venti, Aphanes arvensis, Arabidopsis thaliana, Centaurea cyanus, Elytrigia repens, Poa pratensis, Triticum aestivum, Vicia hirsuta*
3C (herb cover 75%): *Anthemis arvensis, Apera spica-venti, Bromus hordeaceus, Elytrigia repens, Geranium pussilum, Poa pratensis, Polygonum aviculare*
3D (herb cover 50%): *Anthemis arvensis, Apera spica-venti, Aphanes arvensis, Arabidopsis thaliana, Arenaria serpyllifolia, Capsella bursa-pastoris, Centaurea cyanus, Elytrigia repens, Matricaria recutita, Triticum aestivum, Veronica arvensis, Vicia hirsuta*
3E (herb cover 75%): *Apera spica-venti, Artemisia vulgaris, Avena sativa, Centaurea cyanus, Elytrigia repens, Erodium cicutaria, Fallopia convolvulus, Galium aparine, Matricaria recutita, Taraxacum* sect. *Ruderalia, Triticum aestivum, Vicia hirsuta*
4A (herb cover 60%): *Anthemis arvensis, Apera spica-venti, Artemisia vulgaris, Capsella bursa-pastoris, Carum carvi, Cerastium holosteoides, Cirsium arvense, Elytrigia repens, Chaerophyllum aureum, Chenopodium album, Lolium perenne, Lycopsis arvensis, Phleum pratense, Plantago major, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Vicia hirsuta*
4B (herb cover 70%): *Anthemis arvensis, Artemisia vulgaris, Capsella bursa-pastoris, Carum carvi, Galium aparine, Geranium pussilum, Chaerophyllum aureum, Chenopodium album, Leontodon hispidus, Phleum pratense, Plantago major, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Veronica arvensis, Vicia hirsuta, Vicia tetrasperma*
4C (herb cover 90%): *Achillea millefolium, Apera spica-venti, Artemisia vulgaris, Avena sativa, Capsella bursa-pastoris, Convolvulus arvensis, Elytrigia repens, Euphorbia helioscopia, Galeopsis tetrahit, Geranium pussilum, Chaerophyllum aureum, Lolium perenne, Myosotis arvensis, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Veronica arvensis, Viola arvensis*
4D (herb cover 60%): *Artemisia vulgaris, Capsella bursa-pastoris, Galium aparine, Heracleum sphondylium, Chaerophyllum aureum, Leontodon hispidus, Myosotis arvensis, Plantago major, Poa pratensis, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Trifolium repens, Veronica arvensis, Vicia hirsuta, Viola arvensis*
4E (herb cover 45%): *Artemisia vulgaris, Avena sativa, Capsella bursa-pastoris, Carum carvi, Galeopsis tetrahit, Galium aparine, G. spurium, Geranium pussilum, Chaerophyllum aureum, Myosotis arvensis, Taraxacum* sect. *Ruderalia, Viola arvensis*
5A (herb cover 35%): *Acer pseudoplatanus, Anthriscus sylvestris, Fallopia convolvulus, Galeopsis tetrahit, Lapsana communis, Thlaspi arvense, Vicia cracca*
5B (herb cover 25%): *Acer pseudoplatanus, Capsella bursa-pastoris, Fallopia convolvulus, Galeopsis tetrahit, Lapsana communis, Triticum aestivum, Vicia tetrasperma, Viola arvensis*
5C (herb cover 10%): *Acer pseudoplatanus, Fallopia convolvulus, Festuca rubra, Geranium pussilum, Lapsana communis, Triticum aestivum, Vicia tetrasperma, Viola arvensis*
5D (herb cover 50%): *Acer pseudoplatanus, Arabidopsis thaliana, Centaurea cyanus, Elytrigia repens, Galeopsis tetrahit, Geranium pussilum, Lapsana communis, Thlaspi arvense, Vicia tetrasperma, Viola arvensis*
5E (herb cover 50%): *Aethusa cynapium, Agrostis stolonifera, Anthriscus sylvestris, Capsella bursa-pastoris, Centaurea cyanus, Elytrigia repens, Festuca rubra, Galeopsis tetrahit, Geranium pussilum, Lapsana communis, Thlaspi arvense, Triticum aestivum, Vicia tetrasperma, Viola arvensis*
6A (herb cover 30%): *Cirsium arvense, Euphorbia helioscopia, Polygonum aviculare, Scleranthus annuus, Veronica arvensis, V. persica, Vicia cracca, Zea mays*
6B (herb cover 60%): *Centaurea cyanus, Fallopia convolvulus, Polygonum aviculare, Scleranthus annuus, Veronica persica, Viola arvensis, Zea mays*

6C (herb cover 80%): *Anthemis arvensis, Apera spica-venti, Centaurea cyanus, Cirsium arvense, Crepis biennis, Fallopia convolvulus, Fumaria officinalis, Galeopsis tetrahit, Hypericum perforatum, Chaerophyllum aureum, Chenopodium album, Triticum aestivum, Veronica persica, Vicia cracca*

6D (herb cover 50%): *Apera spica-venti, Arrhenatherum elatius, Artemisia vulgaris, Dactylis glomerata, Hypericum perforatum, Chenopodium album, Lapsana communis, Rubus caesius, Scleranthus annuus, Spergula arvensis, Veronica persica, Vicia tetrasperma*

6E (herb cover 50%): *Anagallis arvensis, Arrhenatherum elatius, Convolvulus arvensis, Festuca rubra, Galeopsis tetrahit, Chenopodium album, Knautia arvensis, Linaria vulgaris, Lycopsis arvensis, Myosotis arvensis, Rubus caesius, Stellaria media, Triticum aestivum, Vicia cracca*

7A (herb cover 70%): *Anagallis arvensis, Brassica napus, Capsella bursa-pastoris, Epilobium tetragonum, Fallopia convolvulus, Matricaria recutita, Myosotis arvensis, Plantago major, Poa trivialis, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Veronica persica, Viola arvensis*

7B (herb cover 85%): *Brassica napus, Capsella bursa-pastoris, Fallopia convolvulus, Fumaria officinalis, Galium aparine, Lycopsis arvensis, Matricaria recutita, Papaver rhoeas, P. somniferum, Plantago major, Polygonum aviculare, Thlaspi arvense, Veronica persica*

7C (herb cover 60%): *Atriplex patula, Bromus hordeaceus, Epilobium tetragonum, Euphorbia helioscopia, Galium aparine, Poa trivialis, Polygonum aviculare, Triticum aestivum, Veronica persica*

7D (herb cover 90%): *Anagallis arvensis, Epilobium tetragonum, Fallopia convolvulus, Fumaria officinalis, Galium aparine, Lycopsis arvensis, Matricaria recutita, Myosotis arvensis, Poa trivialis, Polygonum aviculare, Thlaspi arvense, Veronica persica*

7E (herb cover 80%): *Atriplex patula, Fumaria officinalis, Plantago major, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Veronica persica, Viola arvensis*

8A (herb cover 60%): *Aethusa cynapium, Anthemis arvensis, Arenaria serpyllifolia, Brassica napus, Capsella bursa-pastoris, Centaurea cyanus, Galeopsis tetrahit, Hypericum perforatum, Lycopsis arvensis, Matricaria discoidea, Myosotis arvensis, Papaver dubium, Polygonum aviculare, Scleranthus annuus, Stellaria graminea, S. media, Viola arvensis*

8B (herb cover 55%): *Aethusa cynapium, Anthemis arvensis, Arabidopsis thaliana, Arenaria serpyllifolia, Brassica napus, Capsella bursa-pastoris, Centaurea cyanus, Euphorbia helioscopia, Galium aparine, Lapsana communis, Lycopsis arvensis, Myosotis arvensis, Papaver argemone, P. rhoeas, Scleranthus annuus, Stellaria graminea, S. media, Veronica persica, Viola arvensis*

8C (herb cover 70%): *Aethusa cynapium, Anthemis arvensis, Apera spica-venti, Arabidopsis thaliana, Arenaria serpyllifolia, Brassica napus, Capsella bursa-pastoris, Lapsana communis, Lycopsis arvensis, Matricaria discoidea, Myosotis arvensis, Polygonum aviculare, Scleranthus annuus, Stellaria graminea, S. media, Veronica arvensis, V. persica, Vicia tetrasperma, Viola arvensis*

8D (herb cover 70%): *Aethusa cynapium, Anthemis arvensis, Arabidopsis thaliana, Arenaria serpyllifolia, Brassica napus, Capsella bursa-pastoris, Centaurea cyanus, Lycopsis arvensis, Myosotis arvensis, Polygonum aviculare, Scleranthus annuus, Thlaspi arvense, Valerianella dentata, Viola arvensis*

8E (herb cover 75%): *Aethusa cynapium, Anagallis arvensis, Anthemis arvensis, Aphanes arvensis, Arabidopsis thaliana, Arenaria serpyllifolia, Capsella bursa-pastoris, Geum urbanum, Lapsana communis, Myosotis arvensis, Papaver argemone, P. rhoeas, Scleranthus annuus, Stellaria graminea, S. media, Vicia tetrasperma, Viola arvensis*

9A (herb cover 95%): *Aethusa cynapium, Anagallis arvensis, Anthemis arvensis, Apera spica-venti, Capsella bursa-pastoris, Cirsium arvense, Elytrigia repens, Geranium pussilum, Hordeum vulgare, Lolium multiflorum, Matricaria recutita, Triticum aestivum, Veronica arvensis, V. persica, Viola arvensis*

9B (herb cover 80%): *Aethusa cynapium, Anagallis arvensis, Anthemis arvensis, Capsella bursa-pastoris, Fallopia convolvulus, Geranium pussilum, Hordeum vulgare, Matricaria recutita, Thlaspi arvense, Veronica persica, Viola arvensis*

9C (herb cover 60%): *Aethusa cynapium, Anagallis arvensis, Anthemis arvensis, Apera spica-venti, Capsella bursa-pastoris, Euphorbia helioscopia, Fallopia convolvulus, Geranium pussilum, Hordeum vulgare, Matricaria discoidea, Myosotis arvensis, Viola arvensis*

9D (herb cover 70%): *Aethusa cynapium, Achillea millefolium, Arrhenatherum elatius, Bromus hordeaceus, Capsella bursa-pastoris, Cirsium arvense, Convolvulus arvensis, Fallopia convolvulus, Festuca rubra, Hordeum vulgare, Hypericum perforatum, Lapsana communis, Poa pratensis, Rubus caesius, Thlaspi arvense, Triticum aestivum, Vicia sativa* agg., *Viola arvensis*

9E (herb cover 50%): *Aethusa cynapium, Anthemis arvensis, Arabidopsis thaliana, Capsella bursa-pastoris, Elytrigia repens, Fallopia convolvulus, Fumaria officinalis, Geranium pussilum, Hordeum vulgare, Linaria vulgaris, Papaver rhoeas, Thlaspi arvense, Viola arvensis*

10A (herb cover 95%): *Aethusa cynapium, Apera spica-venti, Capsella bursa-pastoris, Centaurea cyanus, Euphorbia helioscopia, Fallopia convolvulus, Fumaria officinalis, Lycopsis arvensis, Matricaria discoidea, M. recutita, Neslia paniculata, Papaver argemone, Polygonum aviculare, Thlaspi arvense, Viola arvensis*

10B (herb cover 90%): *Aethusa cynapium, Capsella bursa-pastoris, Centaurea cyanus, Euphorbia helioscopia, Fallopia convolvulus, Gnaphalium uliginosum, Chenopodium album, Lycopsis arvensis, Matricaria discoidea, M. recutita, Papaver rhoeas, Polygonum aviculare, Viola arvensis*

10C (herb cover 95%): *Aethusa cynapium, Capsella bursa-pastoris, Fallopia convolvulus, Fumaria officinalis, Lycopsis arvensis, Matricaria discoidea, Persicaria maculosa, Urtica dioica, Viola arvensis*

10D (herb cover 90%): *Aethusa cynapium, Capsella bursa-pastoris, Centaurea cyanus, Elytrigia repens, Euphorbia helioscopia, Fallopia convolvulus, Fumaria officinalis, Lycopsis arvensis, Persicaria lapathifolia, Viola arvensis*

10E (herb cover 95%): *Aethusa cynapium, Capsella bursa-pastoris, Centaurea cyanus, Fallopia convolvulus, Galeopsis tetrahit, Lycopsis arvensis, Matricaria discoidea, M. recutita, Papaver rhoeas, Polygonum aviculare, Thlaspi arvense, Veronica persica, Viola arvensis*

11A (herb cover 50%): *Agrostis stolonifera, Arrhenatherum elatius, Bromus hordeaceus, Dactylis glomerata, Chenopodium album, Leontodon autumnalis, Plantago major, Poa pratensis, Puccinelia distans, Spergularia rubra, Taraxacum* sect. *Ruderalia*

11B (herb cover 50%): *Agrostis stolonifera, Achillea millefolium, Anthriscus sylvestris, Apera spica-venti, Bromus hordeaceus, Dactylis glomerata, Leontodon autumnalis, Plantago major, Poa pratensis, Spergularia rubra*
11C (herb cover 85%): *Agrostis stolonifera, Anthriscus sylvestris, Apera spica-venti, Artemisia vulgaris, Dactylis glomerata, Leontodon autumnalis, Matricaria discoidea, M. recutita, Plantago major, Poa annua, P. pratensis, Spergularia rubra*
11D (herb cover 75%): *Agrostis stolonifera, Cerastium holosteoides, Dactylis glomerata, Festuca arundinacea, Leontodon autumnalis, Lolium perenne, Plantago major, Poa annua, Spergularia rubra, Taraxacum* sect. *Ruderalia*
11E (herb cover 90%): *Agrostis stolonifera, Artemisia vulgaris, Bromus hordeaceus, Cerastium holosteoides, Dactylis glomerata, Leontodon autumnalis, Myosotis arvensis, Taraxacum* sect. *Ruderalia, Urtica dioica*
12A (herb cover 40%): *Dactylis glomerata, Matricaria discoidea, M. recutita, Plantago major, Poa pratensis, Polygonum aviculare, Puccinelia distans, Tanacetum vulgare*
12B (herb cover 30%): *Matricaria discoidea, M. recutita, Plantago major, Poa pratensis*
12C (herb cover 45%): *Agrostis stolonifera, Capsella bursa-pastoris, Carduus acanthoides, Fallopia convolvulus, Chenopodium album, Matricaria discoidea, M. recutita, Plantago lanceolata, P. major, Polygonum aviculare, Veronica persica*
12D (herb cover 50%): *Artemisia vulgaris, Dactylis glomerata, Daucus carota, Fallopia convolvulus, Chenopodium album, Matricaria discoidea, M. recutita, Plantago lanceolata, P. major, Poa annua, P. pratensis, Polygonum aviculare, Veronica persica*
12E (herb cover 45%): *Agrostis stolonifera, Arctium tomentosum, Artemisia vulgaris, Atriplex patula, Capsella bursa-pastoris, Dactylis glomerata, Matricaria discoidea, M. recutita, Melilotus officinalis, Persicaria lapathifolia, Plantago major, Poa annua, Polygonum aviculare, Thlaspi arvense, Veronica persica*
13A (herb cover 65%): *Anthemis arvensis, Apera spica-venti, Bromus hordeaceus, Capsella bursa-pastoris, Erodium cicutaria, Geranium pussilum, Lapsana communis, Matricaria recutita, Triticum aestivum, Vicia hirsuta, V. sativa* agg., *Viola arvensis*
13B (herb cover 60%): *Aethusa cynapium, Anthemis arvensis, Apera spica-venti, Capsella bursa-pastoris, Galeopsis tetrahit, Geranium pussilum, Lapsana communis, Matricaria recutita, Triticum aestivum, Vicia hirsuta, V. sativa* agg., *V. tetrasperma*
13C (herb cover 85%): *Anthemis arvensis, Apera spica-venti, Arabidopsis thaliana, Bromus hordeaceus, Equisetum arvense, Galeopsis tetrahit, Geranium pussilum, Matricaria recutita, Myosotis arvensis, Papaver dubium, Trifolium arvense, Triticum aestivum, Veronica arvensis, Vicia hirsuta, V. sativa* agg., *V. tetrasperma, Viola arvensis*
13D (herb cover 70%): *Anthemis arvensis, Apera spica-venti, Aphanes arvensis, Arabidopsis thaliana, Capsella bursa-pastoris, Geranium pussilum, Lapsana communis, Matricaria recutita, Myosotis arvensis, Papaver dubium, P. rhoeas, Stellaria media, Triticum aestivum, Vicia hirsuta, V. sativa* agg., *Viola arvensis*
13E (herb cover 30%): *Apera spica-venti, Centaurea cyanus, Elytrigia repens, Galium aparine, Lapsana communis, Thlaspi arvense, Triticum aestivum, Vicia hirsuta*
14A (herb cover 65%): *Artemisia vulgaris, Capsella bursa-pastoris, Cerastium holosteoides, Cirsium arvense, Conyza canadensis, Elytrigia repens, Matricaria discoidea, M. recutita, Poa annua, Polygonum aviculare, Rubus caesius, Sonchus oleraceus*
14B (herb cover 60%): *Cirsium arvense, Conyza canadensis, Elytrigia repens, Epilobium tetragonum, Matricaria discoidea, M. recutita, Medicago lupulina, Poa annua, Rubus caesius, Sonchus oleraceus, Veronica persica*
14C (herb cover 55%): *Agrostis stolonifera, Capsella bursa-pastoris, Elytrigia repens, Lolium perenne, Matricaria recutita, Melilotus officinalis, Plantago major, Poa annua, Polygonum aviculare, Trifolium hybridum, Veronica persica*
14D (herb cover 55%): *Capsella bursa-pastoris, Cerastium glomeratum, Conyza canadensis, Lactuca serriola, Matricaria discoidea, Plantago major, Poa annua, Ranunculus repens, Sonchus oleraceus, Trifolium hybridum*
14E (herb cover 25%): *Capsella bursa-pastoris, Conyza canadensis, Geranium pussilum, Lolium perenne, Matricaria discoidea, M. recutita, Melilotus officinalis, Poa annua, P. compressa, Rumex crispus, Taraxacum* sect. *Ruderalia*
15A (herb cover 85%): *Anthemis arvensis, Capsella bursa-pastoris, Conyza canadensis, Dactylis glomerata, Elytrigia repens, Geranium pussilum, Matricaria recutita, Plantago major, Poa palustris, P. trivialis, Ranunculus repens, Stellaria graminea, Symphytum officinale, Urtica dioica, Veronica arvensis, V. chamaedrys*
15B (herb cover 70%): *Agrostis stolonifera, Capsella bursa-pastoris, Conyza canadensis, Galeopsis tetrahit, Matricaria discoidea, Plantago major, Poa palustris, P. trivialis, Polygonum aviculare, Symphytum officinale, Taraxacum* sect. *Ruderalia, Trifolium hybridum, Urtica dioica, Viola arvensis*
15C (herb cover 100%): *Anthemis arvensis, Dactylis glomerata, Elytrigia repens, Galium aparine, Matricaria recutita, Myosotis arvensis, Poa trivialis, Sonchus oleraceus, Stellaria media, Urtica dioica*
15D (herb cover 95%): *Anthemis arvensis, Dactylis glomerata, Elytrigia repens, Epilobium ciliatum, Galium aparine, Matricaria recutita, Myosotis arvensis, Poa palustris, P. trivialis, Urtica dioica*
15E (herb cover 85%): *Apera spica-venti, Aphanes arvensis, Elytrigia repens, Galium aparine, Matricaria recutita, Myosotis arvensis, Poa trivialis, Sonchus oleraceus*
16A (herb cover 60%): *Agrostis stolonifera, Arenaria serpyllifolia, Artemisia vulgaris, Cerastium holosteoides, Cirsium vulgare, Elytrigia repens, Geranium dissectum, G. pussilum, Herniaria glabra, Holcus lanatus, Hypochaeris radicata, Lolium perenne, Lotus corniculatus, Phleum pratense, Plantago lanceolata, Poa trivialis, Taraxacum* sect. *Ruderalia*
16B (herb cover 50%): *Agrostis stolonifera, Arenaria serpyllifolia, Dactylis glomerata, Elytrigia repens, Geranium pussilum, Herniaria glabra, Lotus corniculatus, Phleum pratense, Poa compressa, Taraxacum* sect. *Ruderalia*
16C (herb cover 60%): *Agrostis stolonifera, Achillea millefolium, Arenaria serpyllifolia, Artemisia vulgaris, Cerastium holosteoides, Geranium pussilum, Herniaria glabra, Holcus lanatus, Lolium perenne, Medicago lupulina, Melilotus officinalis, Plantago major, Taraxacum* sect. *Ruderalia*
16D (herb cover 90%): *Agrostis stolonifera, Apera spica-venti, Arenaria serpyllifolia, Artemisia vulgaris, Cerastium holosteoides, Geranium pussilum, Herniaria glabra, Holcus lanatus, Lolium perenne, Lotus corniculatus, Myosotis arvensis, Pilosella* sp., *Taraxacum* sect. *Ruderalia, Trifolium campestre, Tussilago farfara*

16E (herb cover 50%): *Agrostis stolonifera, Achillea millefolium, Cerastium holosteoides, Elytrigia repens, Equisetum arvense, Leucanthemum vulgare, Medicago lupulina, Myosotis arvensis, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Trifolium campestre*
17A (herb cover 55%): *Cirsium palustre, Dactylis glomerata, Daucus carota, Deschampsia cespitosa, Festuca pratensis, Lotus corniculatus, Lupinus polyphyllus, Myosotis arvensis, Pilosella* sp., *Plantago major, Poa compressa, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Trifolium pratense, T. repens*
17B (herb cover 65%): *Agrostis stolonifera, Daucus carota, Lathyrus pratensis, Leontodon autumnalis, Myosotis arvensis, Plantago major, Poa compressa, Prunella vulgaris, Tanacetum vulgare, Trifolium hybridum, T. pratense*
17C (herb cover 80%): *Agrostis stolonifera, Achillea millefolium, Artemisia vulgaris, Carex contigua, Deschampsia cespitosa, Hypericum perforatum, Leontodon autumnalis, Lotus corniculatus, Pilosella* sp., *Poa compressa, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Trifolium hybridum, T. repens*
17D (herb cover 60%): *Agrostis stolonifera, Carex contigua, Cirsium arvense, Daucus carota, Deschampsia cespitosa, Leontodon autumnalis, Lotus corniculatus, Plantago major, Poa compressa, Spergularia rubra, Trifolium hybridum*
17E (herb cover 55%): *Agrostis stolonifera, Leontodon autumnalis, Matricaria discoidea, Plantago major, Poa compressa, Sagina procumbens, Tanacetum vulgare, Trifolium hybridum, T. repens*
18A (herb cover 40%): *Agrostis stolonifera, Arabidopsis thaliana, Bromus hordeaceus, Capsella bursa-pastoris, Carex hirta, Cerastium holosteoides, Dactylis glomerata, Leontodon autumnalis, Phleum pratense, Taraxacum* sect. *Ruderalia*
18B (herb cover 45%): *Dactylis glomerata, Festuca arundinacea, Festuca rubra, Leontodon autumnalis, Lotus corniculatus, Phleum pratense, Poa pratensis, Taraxacum* sect. *Ruderalia*
18C (herb cover 50%): *Agrostis stolonifera, Achillea millefolium, Artemisia vulgaris, Festuca arundinacea, F. rubra, Galium album* agg., *Leontodon autumnalis, Lolium perenne, Lotus corniculatus, Plantago major, Poa pratensis, Rumex acetosella, Trifolium repens, Trisetum flavescens, Veronica officinalis*
18D (herb cover 35%): *Agrostis stolonifera, Achillea millefolium, Artemisia vulgaris, Cerastium holosteoides, Festuca rubra, Leontodon autumnalis, Poa pratensis, Trifolium hybridum, T. repens*
18E (herb cover 35%): *Artemisia vulgaris, Carex hirta, Cerastium holosteoides, Equisetum arvense, Festuca arundinacea, Leontodon autumnalis, Matricaria discoidea, Plantago major, Poa pratensis, Polygonum aviculare, Potentilla anserina*
19A (herb cover 85%): *Agrostis stolonifera, Anthriscus sylvestris, Atriplex patula, Avena sativa, Capsella bursa-pastoris, Centaurea cyanus, Cerastium holosteoides, Elytrigia repens, Fallopia convolvulus, Festuca arundinacea, F. pratensis, Heracleum sphondylium, Chenopodium album, Lamium purpureum, Myosotis arvensis, Papaver rhoeas, Plantago major, Polygonum aviculare, Sonchus oleraceus, Stellaria media, Thlaspi arvense, Veronica arvensis, V. persica*
19B (herb cover 90%): *Artemisia vulgaris, Avena sativa, Capsella bursa-pastoris, Centaurea cyanus, Cerastium holosteoides, Cirsium arvense, Elytrigia repens, Euphorbia helioscopia, Fallopia convolvulus, Chenopodium album, Lathyrus tuberosus, Lolium perenne, Lycopsis arvensis, Matricaria discoidea, Myosotis arvensis, Sonchus oleraceus, Stellaria media, Thlaspi arvense, Urtica dioica, Veronica arvensis, V. persica*
19C (herb cover 95%): *Avena sativa, Capsella bursa-pastoris, Centaurea cyanus, Cirsium arvense, Elytrigia repens, Euphorbia helioscopia, Fallopia convolvulus, Galeopsis tetrahit, Chenopodium album, Lamium purpureum, Matricaria discoidea, Myosotis arvensis, Papaver rhoeas, Polygonum aviculare, Sonchus oleraceus, Thlaspi arvense, Veronica arvensis, V. persica, Vicia tetrasperma, Viola arvensis*
19D (herb cover 75%): *Agrostis stolonifera, Arctium lappa, Atriplex patula, Avena sativa, Capsella bursa-pastoris, Centaurea cyanus, Cerastium holosteoides, Cirsium arvense, Elytrigia repens, Euphorbia helioscopia, Fallopia convolvulus, Fumaria officinalis, Galeopsis tetrahit, Heracleum sphondylium, Chenopodium album, Lamium purpureum, Lycopsis arvensis, Myosotis arvensis, Silene noctiflora, Sonchus oleraceus, Stellaria media, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Veronica arvensis, V. persica, Vicia tetrasperma*
19E (herb cover 90%): *Agrostis stolonifera, Avena sativa, Capsella bursa-pastoris, Centaurea cyanus, Cirsium arvense, Elytrigia repens, Fallopia convolvulus, Galeopsis tetrahit, Chenopodium album, Lamium purpureum, Lycopsis arvensis, Matricaria discoidea, Myosotis arvensis, Poa pratensis, Silene noctiflora, Sonchus oleraceus, Stachys palustris, Stellaria media, Thlaspi arvense, Trifolium repens, Veronica arvensis, V. persica, Vicia hirsuta, V. sativa* agg., *Viola arvensis*
20A (herb cover 60%): *Matricaria discoidea, Myosotis arvensis, Phleum pratense, Plantago major, Poa palustris, Sonchus oleraceus, Trifolium repens, Veronica arvensis, V. serpyllifolia*
20B (herb cover 50%): *Agrostis stolonifera, Deschampsia cespitosa, Juncus buffonius, Matricaria discoidea, Persicaria lapathifolia, Phleum pratense, Plantago major, Poa trivialis, Ranunculus repens, Rumex acetosa, Taraxacum* sect. *Ruderalia, Trifolium repens, Urtica dioica, Veronica serpyllifolia*
20C (herb cover 80%): *Cerastium holosteoides, Epilobium hirsutum, E. tetragonum, Galeopsis tetrahit, Geum urbanum, Chenopodium album, Matricaria discoidea, Phleum pratense, Poa trivialis, Polygonum aviculare, Ranunculus repens, Rumex acetosa, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Trifolium repens*
20D (herb cover 35%): *Epilobium tetragonum, Geum urbanum, Juncus buffonius, Matricaria discoidea, Phleum pratense, Plantago major, Poa trivialis, Polygonum aviculare, Ranunculus repens, Tanacetum vulgare, Trifolium repens, Urtica dioica*
20E (herb cover 60%): *Agrostis stolonifera, Atriplex patula, Cirsium vulgare, Festuca pratensis, Chenopodium album, Phleum pratense, Poa trivialis, Ranunculus repens, Rumex acetosa, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Trifolium hybridum, T. repens, Veronica arvensis, Vicia tetrasperma*
21A (herb cover 95%): *Apera spica-venti, Capsella bursa-pastoris, Galeopsis tetrahit, Galium aparine, Geranium pussilum, Lapsana communis, Myosotis arvensis, Phleum pratense, Poa trivialis, Stellaria media, Symphytum officinale, Veronica arvensis, Vicia hirsuta*
21B (herb cover 85%): *Apera spica-venti, Arctium lappa, Atriplex patula, Capsella bursa-pastoris, Cirsium arvense, Elytrigia repens, Fallopia convolvulus, Galeopsis tetrahit, Galium aparine, Geranium pussilum, Chenopodium album, Matricaria discoidea, Myosotis*

arvensis, Papaver rhoeas, Silene noctiflora, Thlaspi arvense, Triticum aestivum, Urtica dioica, Veronica arvensis, Vicia hirsuta, V. tetrasperma

21C (herb cover 80%): *Anthriscus sylvestris, Cerastium holosteoides, Chenopodium album, Lolium perenne, Persicaria lapathifolia, Phleum pratense, Plantago major, Poa annua, Polygonum aviculare, Rumex acetosa, Trifolium hybridum*

21D (herb cover 75%): *Agrostis stolonifera, Arctium tomentosum, Atriplex patula, Capsella bursa-pastoris, Elytrigia repens, Fallopia convolvulus, Lolium perenne, Matricaria discoidea, Myosotis arvensis, Persicaria lapathifolia, Phleum pratense, Poa annua, Polygonum aviculare, Stellaria media, Trifolium repens, Veronica arvensis, Vicia hirsuta, V. tetrasperma*

21E (herb cover 60%): *Agrostis stolonifera, Apera spica-venti, Arabidopsis thaliana, Capsella bursa-pastoris, Centaurea cyanus, Cirsium arvense, Elytrigia repens, Fallopia convolvulus, Galeopsis tetrahit, Geranium pussilum, Lithospermum arvense, Myosotis arvensis, Odontites vernus, Papaver rhoeas, Phleum pratense, Poa trivialis, Trifolium arvense, T. hybridum, Veronica arvensis, Vicia hirsuta, Viola arvensis*

22A (herb cover 90%): *Agrostis stolonifera, Cerastium holosteoides, Equisetum palustre, Myosotis arvensis, Phleum pratense, Plantago major, Poa pratensis, Polygonum aviculare, Ranunculus repens, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica arvensis, V. serpyllifolia*

22B (herb cover 85%): *Agrostis stolonifera, Anthriscus sylvestris, Cerastium holosteoides, Equisetum palustre, Festuca pratensis, Leontodon autumnalis, Matricaria discoidea, Phleum pratense, Plantago major, Polygonum aviculare, Ranunculus repens, Sonchus oleraceus, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica arvensis, V. serpyllifolia*

22C (herb cover 85%): *Agrostis stolonifera, Anthriscus sylvestris, Atriplex patula, Capsella bursa-pastoris, Cerastium holosteoides, Dactylis glomerata, Equisetum palustre, Chenopodium album, Leontodon autumnalis, Matricaria discoidea, Myosotis arvensis, Phleum pratense, Plantago major, Poa trivialis, Polygonum aviculare, Ranunculus repens, Sonchus oleraceus, Trifolium repens, Veronica arvensis, V. serpyllifolia*

22D (herb cover 95%): *Agrostis stolonifera, Anthriscus sylvestris, Atriplex patula, Capsella bursa-pastoris, Cerastium holosteoides, Dactylis glomerata, Galeopsis tetrahit, Leontodon autumnalis, Matricaria discoidea, Phleum pratense, Plantago lanceolata, P. major, Polygonum aviculare, Sonchus oleraceus, Trifolium repens, Veronica arvensis*

22E (herb cover 95%): *Agrostis stolonifera, Capsella bursa-pastoris, Cerastium holosteoides, Leontodon autumnalis, Mentha arvensis, Myosotis arvensis, Phleum pratense, Plantago major, Polygonum aviculare, Ranunculus acris, R. repens, Sonchus oleraceus, Trifolium repens, Veronica arvensis, Viola arvensis*

23A (herb cover 85%): *Agrostis stolonifera, Alopecurus geniculatus, Cerastium holosteoides, Cirsium arvense, Matricaria discoidea, Phleum pratense, Plantago major, Poa trivialis, Ranunculus repens, Rorippa palustris, Trifolium repens*

23B (herb cover 75%): *Agrostis stolonifera, Cerastium holosteoides, Elytrigia repens, Matricaria discoidea, Phleum pratense, Plantago lanceolata, P. major, Poa annua, Ranunculus repens, Rorippa palustris, Taraxacum* sect. *Ruderalia, Trifolium pratense, T. repens*

23C (herb cover 80%): *Agrostis stolonifera, Alopecurus geniculatus, Matricaria discoidea, Plantago major, Poa annua, P. palustris, Ranunculus repens, Rorippa palustris, Stellaria graminea, Trifolium repens*

23D (herb cover 85%): *Agrostis stolonifera, Alopecurus geniculatus, Cerastium holosteoides, Elytrigia repens, Glyceria fluitans, Matricaria discoidea, Plantago major, Poa trivialis, Ranunculus repens, Rorippa palustris, Stellaria graminea, Taraxacum* sect. *Ruderalia, Trifolium repens*

23E (herb cover 75%): *Agrostis stolonifera, Alopecurus geniculatus, Cerastium holosteoides, Elytrigia repens, Gnaphalium uliginosum, Plantago major, Ranunculus repens, Trifolium repens*

24A (herb cover 75%): *Brassica napus, Capsella bursa-pastoris, Dactylis glomerata, Elytrigia repens, Geranium dissectum, Matricaria recutita, Plantago major, Poa annua, P. trivialis, Trifolium repens*

24B (herb cover 70%): *Atriplex patula, Matricaria discoidea, Persicaria lapathifolia, Plantago major, Poa annua, Polygonum aviculare, Trifolium pratense, T. repens, Triticum aestivum*

24C (herb cover 95%): *Brassica napus, Matricaria discoidea, Plantago major, Poa annua, Ranunculus repens, Trifolium pratense, Triticum aestivum*

24D (herb cover 95%): *Apera spica-venti, Brassica napus, Capsella bursa-pastoris, Elytrigia repens, Euphorbia helioscopia, Lapsana communis, Matricaria discoidea, Plantago major, Poa annua, Trifolium repens, Triticum aestivum, Veronica persica, Viola arvensis*

24E (herb cover 95%): *Brassica napus, Galeopsis tetrahit, Hordeum vulgare, Lolium perenne, Matricaria discoidea, M. recutita, Plantago major, Poa annua, Trifolium hybridum, T. pratense, Triticum aestivum, Veronica arvensis*

25A (herb cover 90%): *Agrostis stolonifera, Alopecurus aequalis, Matricaria discoidea, Myosotis arvensis, Plantago major, Poa annua, Polygonum aviculare, Ranunculus repens, Trifolium hybridum, T. repens*

25B (herb cover 95%): *Cerastium holosteoides, Lolium perenne, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare, Potentilla anserina, Ranunculus repens, Trifolium hybridum, T. repens*

25C (herb cover 95%): *Acer pseudoplatanus, Agrostis stolonifera, Capsella bursa-pastoris, Chenopodium album, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare, Ranunculus repens, Stellaria media, Trifolium hybridum, T. repens, Trisetum flavescens, Triticum aestivum, Veronica chamaedrys*

25D (herb cover 95%): *Agrostis stolonifera, Bidens frondosa, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare, Potentilla anserina, Ranunculus repens, Trifolium repens, Vicia sativa* agg., *Viola arvensis*

25E (herb cover 95%): *Agrostis stolonifera, Festuca pratensis, Lolium perenne, Matricaria discoidea, Phleum pratense, Plantago major, Poa annua, P. trivialis, Polygonum aviculare, Potentilla anserina, Ranunculus repens, Trifolium repens*

26A (herb cover 95%): *Agrostis stolonifera, Glyceria notana, Persicaria lapathifolia, P. maculosa, Poa annua, P. trivialis, Ranunculus repens, Trifolium pratense, Urtica dioica, Veronica beccabunga*

26B (herb cover 100%): *Agrostis stolonifera, Alopecurus pratensis, Elytrigia repens, Matricaria discoidea, Myosoton aquaticum, Plantago major, Poa annua, P. trivialis, Polygonum aviculare, Ranunculus repens, Urtica dioica*
26C (herb cover 95%): *Agrostis stolonifera, Artemisia vulgaris, Cirsium arvense, Dactylis glomerata, Elytrigia repens, Lolium perenne, Matricaria discoidea, Plantago lanceolata, P. major, Poa pratensis, Ranunculus repens, Stellaria graminea, Taraxacum* sect. *Ruderalia, Trifolium hybridum, T. repens*
26D (herb cover 95%): *Agrostis stolonifera, Cirsium vulgare, Elytrigia repens, Epilobium tetragonum, Gnaphalium uliginosum, Juncus buffonius, Matricaria discoidea, Poa annua, P. trivialis, Polygonum aviculare, Ranunculus repens, Rumex obtusifolius, Trifolium repens, Veronica beccabunga, V. serpyllifolia, Vicia tetrasperma*
26E (herb cover 90%): *Agrostis stolonifera, Cirsium arvense, Elytrigia repens, Plantago major, Poa annua, Polygonum aviculare, Ranunculus repens, Stellaria graminea, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica serpyllifolia*
27A (herb cover 80%): *Capsella bursa-pastoris, Cirsium arvense, Elytrigia repens, Fallopia convolvulus, Galeopsis tetrahit, Lolium perenne, Matricaria discoidea, M. recutita, Mentha arvensis, Myosotis arvensis, Persicaria lapathifolia, Plantago lanceolata, P. major, Potentilla anserina, Ranunculus repens, Spergula arvensis, Stellaria media, Trifolium pratense, Veronica persica, Vicia tetrasperma, Viola arvensis*
27B (herb cover 90%): *Capsella bursa-pastoris, Cerastium holosteoides, Cirsium arvense, Elytrigia repens, Fallopia convolvulus, Galeopsis tetrahit, Gnaphalium uliginosum, Lolium perenne, Matricaria discoidea, M. recutita, Mentha arvensis, Myosotis arvensis, Polygonum aviculare, Ranunculus repens, Spergula arvensis, Stellaria media, Trifolium pratense, T. repens, Veronica arvensis, V. persica, Viola arvensis*
27C (herb cover 95%): *Capsella bursa-pastoris, Elytrigia repens, Fallopia convolvulus, Galeopsis tetrahit, Lolium perenne, Matricaria discoidea, M. recutita, Myosotis arvensis, Polygonum aviculare, Potentilla anserina, Ranunculus repens, Spergula arvensis, Stellaria media, Trifolium pratense, T. repens, Veronica persica, Vicia tetrasperma, Viola arvensis*
27D (herb cover 75%): *Capsella bursa-pastoris, Elytrigia repens, Galeopsis tetrahit, Holcus lanatus, Matricaria discoidea, Myosotis arvensis, Persicaria lapathifolia, Plantago lanceolata, P. major, Potentilla anserina, Ranunculus repens, Sinapis arvensis, Spergula arvensis, Stellaria graminea, S. media, Trifolium pratense, Veronica arvensis, V. persica, Vicia tetrasperma, Viola arvensis*
27E (herb cover 90%): *Agrostis capillaris, Cerastium holosteoides, Festuca pratensis, Galeopsis tetrahit, Lolium perenne, Mentha arvensis, Myosotis arvensis, Plantago lanceolata, Potentilla anserina, Ranunculus repens, Spergula arvensis, Stellaria graminea, S. media, Taraxacum* sect. *Ruderalia, Trifolium hybridum, T. pratense, T. repens, Veronica persica, Vicia cracca, V. tetrasperma*
28A (herb cover 60%): *Apera spica-venti, Capsella bursa-pastoris, Cerastium holosteoides, Epilobium tetragonum, Fallopia convolvulus, Festuca rubra, Galeopsis tetrahit, Hordeum vulgare, Myosotis arvensis, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Triticum aestivum, Veronica arvensis, V. persica, Viola arvensis*
28B (herb cover 80%): *Apera spica-venti, Capsella bursa-pastoris, Cerastium holosteoides, Epilobium tetragonum, Festuca rubra, Galeopsis tetrahit, Hordeum vulgare, Myosotis arvensis, Poa annua, Polygonum aviculare, Triticum aestivum, Veronica arvensis, V. persica, Viola arvensis*
28C (herb cover 50%): *Aethusa cynapium, Capsella bursa-pastoris, Epilobium tetragonum, Fallopia convolvulus, Festuca rubra, Galeopsis tetrahit, Hordeum vulgare, Matricaria discoidea, Myosotis arvensis, Persicaria maculosa, Poa annua, Veronica arvensis, Viola arvensis*
28D (herb cover 70%): *Apera spica-venti, Aphanes arvensis, Capsella bursa-pastoris, Cerastium holosteoides, Epilobium tetragonum, Euphorbia helioscopia, Fallopia convolvulus, Festuca rubra, Galeopsis tetrahit, Hordeum vulgare, Hypericum perforatum, Myosotis arvensis, Poa annua, Polygonum aviculare, Spergula arvensis, Taraxacum* sect. *Ruderalia, Veronica arvensis, V. persica, Viola arvensis*
28E (herb cover 80%): *Aethusa cynapium, Apera spica-venti, Aphanes arvensis, Capsella bursa-pastoris, Euphorbia helioscopia, Fallopia convolvulus, Galeopsis tetrahit, Hypericum perforatum, Chenopodium album, Myosotis arvensis, Persicaria maculosa, Poa annua, Polygonum aviculare, Sagina procumbens, Taraxacum* sect. *Ruderalia, Veronica arvensis, V. persica, Viola arvensis*
29A (herb cover 95%): *Atriplex patula, Dactylis glomerata, Matricaria discoidea, Myosotis arvensis, Myosoton aquaticum, Plantago major, Poa annua, Polygonum aviculare, Stellaria media, Trifolium repens*
29B (herb cover 95%): *Capsella bursa-pastoris, Elytrigia repens, Lolium perenne, Matricaria discoidea, Myosotis arvensis, Myosoton aquaticum, Plantago major, Poa annua, P. trivialis, Polygonum aviculare, Stellaria media, Taraxacum* sect. *Ruderalia, Trifolium pratense, T. repens, Vicia sativa* agg., *Viola arvensis*
29C (herb cover 90%): *Elytrigia repens, Galeopsis tetrahit, Chenopodium album, Matricaria discoidea, Myosotis arvensis, Poa annua, P. trivialis, Sonchus oleraceus, Stellaria media, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica arvensis, Viola arvensis*
29D (herb cover 95%): *Agrostis stolonifera, Achillea millefolium, Artemisia vulgaris, Cerastium holosteoides, Elytrigia repens, Plantago major, Poa annua, P. trivialis, Rorippa palustris, Stellaria graminea, S. media, Trifolium repens*
29E (herb cover 95%): *Agrostis stolonifera, Cerastium holosteoides, Dactylis glomerata, Elytrigia repens, Matricaria discoidea, Plantago major, Poa annua, P. trivialis, Ranunculus repens, Rumex obtusifolius, Stellaria media, Trifolium pratense, T. repens, Vicia tetrasperma*
30A (herb cover 95%): *Agrostis stolonifera, Alchemilla* sp., *Atriplex patula, Capsella bursa-pastoris, Cerastium holosteoides, Dactylis glomerata, Elytrigia repens, Epilobium tetragonum, Fallopia convolvulus, Festuca pratensis, Gnaphalium uliginosum, Juncus buffonius, Lolium perenne, Matricaria discoidea, M. recutita, Plantago major, Poa annua, P. trivialis, Polygonum aviculare, Ranunculus repens, Sagina procumbens, Sonchus oleraceus, Thlaspi arvense, Trifolium repens, Veronica serpyllifolia*
30B (herb cover 85%): *Agrostis stolonifera, Alchemilla* sp., *Alopecurus geniculatus, Atriplex patula, Capsella bursa-pastoris, Cerastium holosteoides, Echinochloa crus-galli, Epilobium ciliatum, Glyceria notana, Lolium perenne, Matricaria discoidea, M. recutita, Persicaria hydropiper, Plantago lanceolata, P. major, Poa annua, P. trivialis, Polygonum aviculare, Prunella vulgaris, Ranunculus repens, Rorippa palustris, Sagina procumbens, Sonchus oleraceus, Trifolium repens, Veronica serpyllifolia*

30C (herb cover 80%): *Agrostis stolonifera, Capsella bursa-pastoris, Epilobium tetragonum, Chenopodium album, Lolium perenne, Matricaria discoidea, M. recutita, Persicaria lapathifolia, Plantago lanceolata, P. major, Poa annua, Polygonum aviculare, Ranunculus repens, Rorippa palustris, Sonchus oleraceus, Stellaria graminea, Trifolium repens*
30D (herb cover 75%): *Agrostis stolonifera, Alchemilla* sp., *Echinochloa crus-galli, Epilobium tetragonum, Lolium perenne, Matricaria discoidea, M. recutita, Phleum pratense, Plantago major, Poa annua, Polygonum aviculare, Potentilla anserina, Prunella vulgaris, Ranunculus repens, Salix* sp., *Trifolium repens*
30E (herb cover 75%): *Agrostis stolonifera, Atriplex patula, Capsella bursa-pastoris, Cerastium holosteoides, Elytrigia repens, Epilobium tetragonum, Lolium perenne, Matricaria recutita, Plantago major, Poa annua, Polygonum aviculare, Ranunculus repens, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica serpyllifolia*
31A (herb cover 95%): *Agrostis stolonifera, Alopecurus geniculatus, Bolboschoenus* sp., *Elytrigia repens, Epilobium ciliatum, E. tetragonum, Galium palustre, Chenopodium polyspermum, Juncus articulatus, Lycopus europaeus, Matricaria recutita, Persicaria maculosa, Ranunculus repens, Rumex crispus, Taraxacum* sect. *Ruderalia, Trifolium hybridum, Triticum aestivum, Urtica dioica*
31B (herb cover 85%): *Anthriscus sylvestris, Artemisia vulgaris, Cirsium arvense, Elytrigia repens, Galeopsis tetrahit, Holcus mollis, Hordeum vulgare, Hypericum perforatum, Lapsana communis, Plantago major, Ranunculus repens, Tanacetum vulgare, Trifolium hybridum, Triticum aestivum, Vicia sativa* agg.
31C (herb cover 85%): *Apera spica-venti, Artemisia vulgaris, Cirsium arvense, Elytrigia repens, Epilobium tetragonum, Galium aparine, Holcus mollis, Hordeum vulgare, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Trifolium hybridum, Triticum aestivum, Vicia tetrasperma*
31D (herb cover 80%): *Apera spica-venti, Arabidopsis thaliana, Artemisia vulgaris, Echinochloa crus-galli, Elytrigia repens, Holcus mollis, Hordeum vulgare, Plantago major, Poa palustris, Trifolium hybridum, Triticum aestivum, Viola arvensis*
31E (herb cover 80%): *Artemisia vulgaris, Atriplex patula, Echinochloa crus-galli, Elytrigia repens, Geranium pussilum, Hordeum vulgare, Matricaria recutita, Plantago major, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Trifolium hybridum, Triticum aestivum, Vicia tetrasperma, Viola arvensis*
32A (herb cover 80%): *Agrostis stolonifera, Anagallis arvensis, Apera spica-venti, Epilobium tetragonum, Hordeum vulgare, Chenopodium polyspermum, Juncus bulbosus, Lolium perenne, Matricaria recutita, Plantago major, Polygonum aviculare, Sagina procumbens, Taraxacum* sect. *Ruderalia*
32B (herb cover 80%): *Agrostis stolonifera, Gnaphalium uliginosum, Hordeum vulgare, Juncus bulbosus, Matricaria recutita, Plantago major, Polygonum aviculare, Sagina procumbens, Vicia tetrasperma, Viola arvensis*
32C (herb cover 85%): *Agrostis stolonifera, Anagallis arvensis, Apera spica-venti, Gnaphalium uliginosum, Hordeum vulgare, Hypericum perforatum, Matricaria discoidea, M. recutita, Myosotis arvensis, Picea abies, Plantago major, Polygonum aviculare, Ranunculus repens, Sonchus asper, Taraxacum* sect. *Ruderalia, Vicia tetrasperma*
32D (herb cover 90%): *Agrostis stolonifera, Anagallis arvensis, Atriplex patula, Elytrigia repens, Epilobium ciliatum, Gnaphalium uliginosum, Hordeum vulgare, Juncus bulbosus, Matricaria recutita, Persicaria hydropiper, Plantago major, Polygonum aviculare, Rorippa palustris, Sagina procumbens, Sonchus asper, Stachys palustris, Taraxacum* sect. *Ruderalia, Vicia tetrasperma, Viola arvensis*
32E (herb cover 95%): *Agrostis stolonifera, Juncus bulbosus, Lolium perenne, Matricaria recutita, Plantago major, Poa annua, Polygonum aviculare, Sagina procumbens, Taraxacum* sect. *Ruderalia, Vicia tetrasperma*
33A (herb cover 95%): *Agrostis stolonifera, Lolium perenne, Pastinaca sativa, Plantago lanceolata, P. major, Poa pratensis, Trifolium hybridum, T. pratense, T. repens, Vicia cracca*
33B (herb cover 85%): *Agrostis stolonifera, Festuca arundinacea, F. pratensis, Lathyrus pratensis, Leontodon hispidus, Lolium perenne, Medicago lupulina, Pastinaca sativa, Plantago lanceolata, Trifolium repens, Vicia cracca, V. tetrasperma*
33C (herb cover 90%): *Agrostis stolonifera, Anagallis arvensis, Cerastium holosteoides, Cirsium arvense, Epilobium tetragonum, Festuca arundinacea, F. pratensis, Lolium perenne, Pastinaca sativa, Plantago lanceolata, P. major, Ranunculus repens, Taraxacum* sect. *Ruderalia, Trifolium repens, Vicia tetrasperma*
33D (herb cover 90%): *Agrostis stolonifera, Cirsium arvense, Daucus carota, Epilobium tetragonum, Festuca arundinacea, Lolium perenne, Pastinaca sativa, Plantago lanceolata, P. major, Ranunculus repens, Taraxacum* sect. *Ruderalia, Trifolium hybridum, T. repens, Vicia cracca, V. tetrasperma*
33E (herb cover 80%): *Agrostis stolonifera, Cerastium holosteoides, Cirsium arvense, Epilobium tetragonum, Festuca arundinacea, Holcus lanatus, Lolium perenne, Mentha arvensis, Phleum pratense, Plantago lanceolata, Ranunculus repens, Trifolium repens, Vicia tetrasperma*
34A (herb cover 85%): *Alchemilla* sp., *Artemisia vulgaris, Capsella bursa-pastoris, Centaurea jacea, Cerastium holosteoides, Dactylis glomerata, Equisetum arvense, Galeopsis tetrahit, Holcus mollis, Lolium perenne, Matricaria discoidea, Plantago lanceolata, P. major, Polygonum aviculare, Ranunculus repens, Rubus caesius, Scleranthus annuus, Sonchus oleraceus, Spergula arvensis, Stellaria graminea, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica arvensis, V. serpyllifolia*
34B (herb cover 90%): *Achillea millefolium, Anagallis arvensis, Capsella bursa-pastoris, Chenopodium album, Lolium perenne, Lupinus polyphyllus, Matricaria discoidea, Myosotis arvensis, Phleum pratense, Plantago lanceolata, P. major, Poa annua, Polygonum aviculare, Rubus caesius, Scleranthus annuus, Spergula arvensis, Stellaria graminea, Taraxacum* sect. *Ruderalia, Trifolium medium, T. repens, Veronica serpyllifolia, Vicia cracca, Viola arvensis*
34C (herb cover 85%): *Anagallis arvensis, Capsella bursa-pastoris, Lolium perenne, Matricaria discoidea, Plantago lanceolata, P. major, Poa annua, Polygonum aviculare, Scleranthus annuus, Spergula arvensis, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Trifolium medium, T. repens, Veronica serpyllifolia, Viola arvensis*
34D (herb cover 95%): *Agrostis stolonifera, Artemisia vulgaris, Cerastium holosteoides, Elytrigia repens, Equisetum arvense, Leucanthemum vulgare, Lolium perenne, Lupinus polyphyllus, Matricaria discoidea, Myosotis arvensis, Myosoton aquaticum, Phleum pratense, Plantago lanceolata, P. major, Poa annua, Polygonum aviculare, Ranunculus repens, Rubus caesius, Scleranthus annuus,*

Spergula arvensis, Stellaria graminea, Taraxacum sect. *Ruderalia, Trifolium medium, T. repens, Veronica arvensis, V. serpyllifolia, Vicia sativa* agg.
34E (herb cover 80%): *Agrostis capillaris, Alchemilla* sp., *Capsella bursa-pastoris, Equisetum arvense, Lolium perenne, Matricaria discoidea, Plantago lanceolata, P. major, Poa annua, Polygonum aviculare, Rubus caesius, Scleranthus annuus, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Trifolium arvense, T. repens, Veronica arvensis, V. serpyllifolia*
35A (herb cover 95%): *Anthemis arvensis, Capsella bursa-pastoris, Elytrigia repens, Galium album* agg., *Geranium pussilum, Lolium perenne, Matricaria discoidea, Plantago lanceolata, P. major, Poa annua, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Trifolium dubium, T. repens, Veronica persica, Vicia sativa* agg., *Vicia tetrasperma*
35B (herb cover 95%): *Achillea millefolium, Anthemis arvensis, Capsella bursa-pastoris, Elytrigia repens, Geranium pussilum, Lolium perenne, Matricaria discoidea, Plantago lanceolata, P. major, Poa annua, Polygonum aviculare, Trifolium repens, Veronica persica*
35C (herb cover 95%): *Achillea millefolium, Capsella bursa-pastoris, Cerastium holosteoides, Geranium pussilum, Chenopodium album, Lolium perenne, Matricaria discoidea, Plantago lanceolata, P. major, Poa annua, Polygonum aviculare, Ranunculus repens, Rumex obtusifolius, Trifolium repens*
35D (herb cover 75%): *Anthemis arvensis, Capsella bursa-pastoris, Dactylis glomerata, Geranium pussilum, Lolium perenne, Malva neglecta, Matricaria discoidea, Plantago lanceolata, P. major, Poa annua, Polygonum aviculare, Rumex obtusifolius, Trifolium dubium, T. repens*
35E (herb cover 80%): *Achillea millefolium, Capsella bursa-pastoris, Geranium pussilum, Lolium perenne, Matricaria discoidea, Plantago lanceolata, P. major, Poa annua, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Trifolium dubium, Veronica persica*
36A (herb cover 55%): *Achillea millefolium, Bromus hordeaceus, Conyza canadensis, Dactylis glomerata, Medicago lupulina, Plantago lanceolata, Poa compressa, Spergularia rubra, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Trifolium hybridum*
36B (herb cover 65%): *Achillea millefolium, Artemisia vulgaris, Bromus hordeaceus, B. tectorum, Geranium pussilum, Plantago lanceolata, Taraxacum* sect. *Ruderalia*
36C (herb cover 75%): *Artemisia vulgaris, Bromus hordeaceus, B. tectorum, Geranium pussilum, Heracleum mantegazzianum, Lathyrus sylvestris, Lepidium campestre, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Trifolium hybridum*
36D (herb cover 65%): *Artemisia vulgaris, Bromus hordeaceus, B. tectorum, Conyza canadensis, Linaria vulgaris, Plantago lanceolata, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Trifolium hybridum*
36E (herb cover 80%): *Artemisia vulgaris, Bromus hordeaceus, B. tectorum, Conyza canadensis, Geranium pussilum, Linaria vulgaris, Poa pratensis, Rubus caesius, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Ulmus scabra*
37A (herb cover 55%): *Bidens frondosa, B. radiata, Chenopodium album, Myosoton aquaticum, Oenanthe aquatica, Persicaria hydropiper, Persicaria lapathifolia, Polygonum aviculare, Stellaria media*
37B (herb cover 65%): *Bidens radiata, Elytrigia repens, Chenopodium album, Myosoton aquaticum, Persicaria lapathifolia, Phleum pratense, Rorippa palustris*
37C (herb cover 70%): *Apera spica-venti, Bidens radiata, Elytrigia repens, Chenopodium album, Lycopus europaeus, Myosoton aquaticum, Oenanthe aquatica, Persicaria lapathifolia, P. maculosa, Poa annua, Rumex maritimus, Taraxacum* sect. *Ruderalia, Thlaspi arvense*
37D (herb cover 80%): *Apera spica-venti, Atriplex patula, Capsella bursa-pastoris, Cirsium vulgare, Echinochloa crus-galli, Elytrigia repens, Epilobium parviflorum, Chenopodium album, Myosoton aquaticum, Oenanthe aquatica, Persicaria lapathifolia, Phleum pratense, Plantago major, Poa annua, Polygonum aviculare, Ranunculus repens, Rumex maritimus*
37E (herb cover 80%): *Agrostis stolonifera, Atriplex patula, Chenopodium album, Myosoton aquaticum, Persicaria lapathifolia, Phleum pratense, Plantago major, Poa annua, Polygonum aviculare, Ranunculus repens, Taraxacum* sect. *Ruderalia, Trifolium hybridum*
38A (herb cover 30%): *Achillea millefolium, Anagallis arvensis, Anthriscus sylvestris, Apera spica-venti, Avena fatua, Bromus hordeaceus, Cirsium arvense, Elytrigia repens, Epilobium tetragonum, Hordeum vulgare, Lolium perenne, Matricaria discoidea, M. recutita, Myosotis arvensis, Plantago major, Poa annua, Polygonum aviculare, Sonchus asper, Sonchus oleraceus, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica arvensis, V. persica, Vicia tetrasperma*
38B (herb cover 40%): *Alchemilla* sp., *Anagallis arvensis, Anthriscus sylvestris, Artemisia vulgaris, Bromus hordeaceus, Dactylis glomerata, Elytrigia repens, Euphorbia helioscopia, Galeopsis tetrahit, Galium aparine, Heracleum sphondylium, Lapsana communis, Leontodon autumnalis, Lolium perenne, Matricaria discoidea, M. recutita, Myosotis arvensis, Plantago lanceolata, Sonchus oleraceus, Taraxacum* sect. *Ruderalia, Veronica arvensis, Vicia cracca, V. hirsuta, V. tetrasperma*
38C (herb cover 80%): *Alopecurus pratensis, Anagallis arvensis, Bromus hordeaceus, Epilobium tetragonum, Euphorbia helioscopia, Geranium pussilum, Hypericum perforatum, Lamium purpureum, Lolium perenne, Matricaria recutita, Myosotis arvensis, Plantago lanceolata, P. major, Ranunculus repens, Sonchus oleraceus, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica arvensis, Vicia cracca, V. hirsuta, V. tetrasperma*
38D (herb cover 80%): *Anagallis arvensis, Apera spica-venti, Artemisia vulgaris, Capsella bursa-pastoris, Elytrigia repens, Fallopia convolvulus, Lamium amplexicaule, Lolium perenne, Matricaria recutita, Myosotis arvensis, Papaver dubium, Plantago lanceolata, P. major, Poa annua, P. pratensis, Polygonum aviculare, Sonchus asper, Sorbus aucuparia, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica arvensis, V. persica, Viola arvensis*
38E (herb cover 65%): *Alopecurus geniculatus, Anagallis arvensis, Avena fatua, Cirsium arvense, Euphorbia helioscopia, Galium aparine, Hordeum vulgare, Chenopodium album, Leontodon autumnalis, Lolium perenne, Matricaria recutita, Myosotis arvensis, Plantago major, Poa annua, Polygonum aviculare, Sorbus aucuparia, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica arvensis, V. persica, Viola arvensis*

39A (herb cover 70%): *Artemisia vulgaris, Atriplex patula, Elytrigia repens, Equisetum arvense, Chenopodium album, Leontodon autumnalis, Polygonum aviculare, Senecio viscosus, Sonchus oleraceus*
39B (herb cover 65%): *Artemisia vulgaris, Cirsium arvense, Elytrigia repens, Equisetum arvense, Chenopodium album, Senecio viscosus, Sonchus arvensis, S. oleraceus*
39C (herb cover 65%): *Achillea millefolium, Artemisia vulgaris, Atriplex patula, Avenula pubescens, Elytrigia repens, Leontodon autumnalis, Polygonum aviculare, Puccinelia distans, Sonchus arvensis, S. oleraceus, Vicia tetrasperma*
39D (herb cover 65%): *Artemisia vulgaris, Avenula pubescens, Elytrigia repens, Chenopodium album, Leontodon autumnalis, Plantago lanceolata, Polygonum aviculare, Senecio viscosus, Sonchus arvensis, S. oleraceus, Taraxacum* sect. *Ruderalia*
39E (herb cover 70%): *Achillea millefolium, Atriplex patula, Avenula pubescens, Elytrigia repens, Festuca arundinacea, Chenopodium album, Leontodon autumnalis, Polygonum aviculare, Spergularia rubra*
40A (herb cover 80%): *Anthemis arvensis, Avena sativa, Capsella bursa-pastoris, Centaurea cyanus, Geranium pussilum, Matricaria recutita, Plantago major, Polygonum aviculare, Secale cereale, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica persica, Viola arvensis*
40B (herb cover 90%): *Anthemis arvensis, Artemisia vulgaris, Centaurea cyanus, Elytrigia repens, Fallopia convolvulus, Galeopsis tetrahit, Geranium pussilum, Holcus mollis, Lycopsis arvensis, Matricaria recutita, Myosotis arvensis, Poa trivialis, Polygonum aviculare, Secale cereale, Taraxacum* sect. *Ruderalia, Veronica persica, Vicia hirsuta*
40C (herb cover 95%): *Capsella bursa-pastoris, Elytrigia repens, Matricaria recutita, Poa annua, P. palustris, Potentilla argentea, Salix* sp., *Silene latifolia, Taraxacum* sect. *Ruderalia, Vicia hirsuta, V. sativa* agg.
40D (herb cover 80%): *Capsella bursa-pastoris, Elytrigia repens, Geranium pussilum, Matricaria recutita, Poa annua, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Veronica arvensis, V. persica, Viola arvensis*
40E (herb cover 75%): *Capsella bursa-pastoris, Elytrigia repens, Geranium pussilum, Holcus mollis, Matricaria recutita, Myosotis arvensis, Plantago lanceolata, P. major, Taraxacum* sect. *Ruderalia*
41A (herb cover 70%): *Agrostis stolonifera, Anagallis arvensis, Aphanes arvensis, Capsella bursa-pastoris, Fallopia convolvulus, Galeopsis tetrahit, Hordeum vulgare, Hypericum perforatum, Lamium purpureum, Matricaria recutita, Plantago major, Poa annua, Polygonum aviculare, Veronica arvensis, Vicia hirsuta, V. sativa* agg., *V. tetrasperma, Viola arvensis*
41B (herb cover 75%): *Agrostis capillaris, Anagallis arvensis, Aphanes arvensis, Artemisia vulgaris, Capsella bursa-pastoris, Fallopia convolvulus, Geranium pussilum, Hordeum vulgare, Hypericum perforatum, Lamium purpureum, Plantago major, Poa annua, Polygonum aviculare, Sonchus asper, Veronica arvensis, V. persica, Vicia hirsuta, V. sativa* agg., *Viola arvensis*
41C (herb cover 75%): *Anagallis arvensis, Aphanes arvensis, Arabidopsis thaliana, Artemisia vulgaris, Capsella bursa-pastoris, Fallopia convolvulus, Hordeum vulgare, Lamium purpureum, Matricaria recutita, Myosotis arvensis, Plantago major, Poa annua, Polygonum aviculare, Sonchus asper, Spergula arvensis, Veronica arvensis, V. persica, Vicia sativa* agg., *Viola arvensis*
41D (herb cover 75%): *Anagallis arvensis, Anthemis arvensis, Apera spica-venti, Aphanes arvensis, Avena sativa, Capsella bursa-pastoris, Dactylis glomerata, Fallopia convolvulus, Galium aparine, Geranium pussilum, Hordeum vulgare, Lapsana communis, Myosotis arvensis, Plantago major, Poa annua, Polygonum aviculare, Spergula arvensis, Thlaspi arvense, Vicia hirsuta, V. sativa* agg., *Viola arvensis*
41E (herb cover 65%): *Anagallis arvensis, Artemisia vulgaris, Fallopia convolvulus, Hordeum vulgare, Matricaria recutita, Plantago major, Poa annua, Rumex acetosa, Veronica arvensis, V. persica, Vicia sativa* agg., *Viola arvensis*
42A (herb cover 90%): *Agrostis stolonifera, Alopecurus geniculatus, Cerastium holosteoides, Festuca pratensis, Juncus buffonius, Plantago major, Poa annua, Ranunculus repens, Rumex acetosa, Sagina procumbens, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica serpyllifolia*
42B (herb cover 85%): *Agrostis stolonifera, Alopecurus geniculatus, Cerastium holosteoides, Epilobium tetragonum, Festuca pratensis, Gnaphalium uliginosum, Juncus buffonius, Plantago major, Poa annua, P. pratensis, Polygonum aviculare, Ranunculus repens, Sagina procumbens, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica serpyllifolia*
42C (herb cover 90%): *Agrostis stolonifera, Alopecurus geniculatus, Epilobium tetragonum, Festuca pratensis, Glyceria notana, Gnaphalium uliginosum, Juncus articulatus, J. buffonius, Plantago major, Poa annua, Ranunculus repens, Sagina procumbens, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica serpyllifolia*
42D (herb cover 85%): *Agrostis stolonifera, Alopecurus geniculatus, Deschampsia cespitosa, Festuca pratensis, Gnaphalium uliginosum, Juncus articulatus, J. buffonius, Lolium perenne, Plantago major, Ranunculus repens, Sagina procumbens, Trifolium repens*
42E (herb cover 80%): *Agrostis stolonifera, Alchemilla* sp., *Alopecurus geniculatus, Cerastium holosteoides, Festuca arundinacea, Juncus buffonius, Matricaria discoidea, Plantago major, Poa annua, Ranunculus repens, Sagina procumbens, Sorbus aucuparia, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica serpyllifolia*
43A (herb cover 85%): *Agrostis stolonifera, Atriplex patula, Capsella bursa-pastoris, Carex hirta, Elytrigia repens, Festuca pratensis, Heracleum sphondylium, Holcus mollis, Juncus articulatus, J. compressus, J. tenuis, Leontodon autumnalis, Persicaria hydropiper, Phleum pratense, Plantago lanceolata, P. major, Poa annua, Potentilla anserina, Prunella vulgaris, Ranunculus repens, Sonchus oleraceus, Trifolium pratense*
43B (herb cover 80%): *Achillea millefolium, Artemisia vulgaris, Atriplex patula, Cirsium arvense, Elytrigia repens, Leontodon autumnalis, Lupinus polyphyllus, Plantago lanceolata, P. major, Polygonum aviculare, Potentilla anserina, Prunella vulgaris, Tanacetum vulgare, Trifolium pratense*
43C (herb cover 75%): *Agrostis stolonifera, Leontodon autumnalis, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare, Ranunculus repens, Sagina procumbens, Taraxacum* sect. *Ruderalia, Trifolium repens*
43D (herb cover 100%): *Agrostis stolonifera, Achillea millefolium, Atriplex patula, Cerastium glomeratum, C. holosteoides, Elytrigia repens, Fallopia convolvulus, Festuca pratensis, Galeopsis pubescens, Lupinus polyphyllus, Myosotis palustris, Persicaria hydropiper,*

P. maculosa, Phleum pratense, Plantago lanceolata, P. major, Poa trivialis, Polygonum aviculare, Potentilla anserina, Ranunculus repens, Taraxacum sect. *Ruderalia, Trifolium pratense*

43E (herb cover 100%): *Achillea millefolium, Alopecurus pratensis, Artemisia vulgaris, Bromus hordeaceus, Capsella bursa-pastoris, Cirsium arvense, Elytrigia repens, Festuca pratensis, Galeopsis pubescens, Galium album* agg., *Glechoma hederacea, Holcus mollis, Lathyrus pratensis, Lupinus polyphyllus, Phleum pratense, Plantago lanceolata, Poa trivialis, Polygonum aviculare, Potentilla anserina, Prunella vulgaris, Ranunculus repens, Tanacetum vulgare, Urtica dioica, Vicia cracca*

44A (herb cover 85%): *Artemisia vulgaris, Capsella bursa-pastoris, Dactylis glomerata, Elytrigia repens, Erysimum cheiranthoides, Fallopia convolvulus, Festuca rubra, Galeopsis tetrahit, Galium aparine, Chenopodium album, Lycopsis arvensis, Matricaria discoidea, Potentilla anserina, Urtica dioica*

44B (herb cover 90%): *Achillea millefolium, Artemisia vulgaris, Elytrigia repens, Erysimum cheiranthoides, Fallopia convolvulus, Festuca rubra, Galium aparine, Heracleum sphondylium, Chenopodium album, Lolium perenne, Lycopsis arvensis, Sonchus oleraceus, Thlaspi arvense, Trifolium repens, Urtica dioica, Vicia cracca*

44C (herb cover 70%): *Agrostis stolonifera, Artemisia vulgaris, Capsella bursa-pastoris, Cirsium arvense, Elytrigia repens, Fallopia convolvulus, Galeopsis tetrahit, Chenopodium album, Lolium perenne, Lycopsis arvensis, Matricaria discoidea, Plantago major, Poa annua, P. pratensis, Polygonum aviculare, Spergula arvensis, Stellaria media, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Trifolium repens, Urtica dioica*

44D (herb cover 90%): *Agrostis stolonifera, Artemisia vulgaris, Capsella bursa-pastoris, Cerastium holosteoides, Cirsium arvense, Elytrigia repens, Fallopia convolvulus, Galium aparine, Chenopodium album, Lolium perenne, Lycopsis arvensis, Matricaria discoidea, Myosotis arvensis, Plantago major, Poa annua, P. pratensis, Polygonum aviculare, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Trifolium repens*

44E (herb cover 80%): *Artemisia vulgaris, Capsella bursa-pastoris, Cirsium arvense, Dactylis glomerata, Elytrigia repens, Fallopia convolvulus, Galium aparine, Heracleum sphondylium, Chenopodium album, Lolium perenne, Matricaria discoidea, Phleum pratense, Plantago major, Poa annua, Polygonum aviculare, Sonchus oleraceus, Stellaria media, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Trifolium repens, Urtica dioica, Veronica arvensis, Vicia cracca, Viola arvensis*

45A (herb cover 70%): *Anthriscus sylvestris, Artemisia vulgaris, Festuca pratensis, Lolium perenne, Poa pratensis, Rubus caesius, Taraxacum* sect. *Ruderalia*

45B (herb cover 70%): *Achillea millefolium, Anthriscus sylvestris, Artemisia vulgaris, Crepis biennis, Euphorbia esula, Festuca pratensis, F. rubra, Chenopodium album, Plantago major, Polygonum aviculare, Rubus caesius, Taraxacum* sect. *Ruderalia, Trifolium repens*

45C (herb cover 80%): *Anthriscus sylvestris, Artemisia vulgaris, Dactylis glomerata, Elytrigia repens, Euphorbia esula, Festuca rubra, Leontodon autumnalis, Plantago lanceolata, Poa pratensis, Taraxacum* sect. *Ruderalia, Trisetum flavescens*

45D (herb cover 80%): *Agrostis stolonifera, Achillea millefolium, Anthriscus sylvestris, Dactylis glomerata, Echium vulgare, Elytrigia repens, Galium album* agg., *Plantago lanceolata, P. major, Poa pratensis, Stellaria graminea, Taraxacum* sect. *Ruderalia, Trifolium repens, Vicia cracca*

45E (herb cover 80%): *Anthriscus sylvestris, Arrhenatherum elatius, Artemisia vulgaris, Dactylis glomerata, Festuca pratensis, Phleum pratense, Rubus caesius, Taraxacum* sect. *Ruderalia, Vicia cracca*

46A (herb cover 30%): *Apera spica-venti, Capsella bursa-pastoris, Cerastium holosteoides, Festuca pratensis, F. rubra, Lolium perenne, Matricaria discoidea, Myosotis arvensis, Plantago major, Poa pratensis, Polygonum aviculare, Tanacetum vulgare, Taraxacum* sect. *Ruderalia*

46B (herb cover 75%): *Cerastium holosteoides, Dactylis glomerata, Elytrigia repens, Leontodon autumnalis, Lolium perenne, Matricaria discoidea, Phleum pratense, Plantago major, Polygonum aviculare, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Trifolium pratense*

46C (herb cover 80%): *Achillea millefolium, Anthriscus sylvestris, Cerastium holosteoides, Dactylis glomerata, Lolium perenne, Myosotis arvensis, Plantago major, Poa pratensis, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Trifolium dubium, T. repens, Veronica arvensis*

46D (herb cover 60%): *Alchemilla* sp., *Anthriscus sylvestris, Atriplex patula, Cerastium holosteoides, Dactylis glomerata, Daucus carota, Plantago major, Poa annua, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Trifolium pratense, T. repens*

46E (herb cover 65%): *Anthriscus sylvestris, Cerastium holosteoides, Dactylis glomerata, Elytrigia repens, Heracleum mantegazzianum, Leontodon autumnalis, Matricaria discoidea, Poa annua, P. pratensis, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Tragopogon orientalis, Trifolium repens*

47A (herb cover 85%): *Agrostis stolonifera, Artemisia vulgaris, Cerastium glomeratum, Cirsium arvense, Epilobium tetragonum, Equisetum arvense, Galeopsis bifida, Glyceria notana, Gnaphalium uliginosum, Juncus buffonius, Matricaria discoidea, Myosoton aquaticum, Plantago major, Poa annua, Polygonum aviculare, Ranunculus repens, Stellaria media, Trifolium repens, Veronica arvensis, V. serpyllifolia*

47B (herb cover 70%): *Agrostis stolonifera, Artemisia vulgaris, Epilobium ciliatum, Equisetum arvense, Glyceria notana, Gnaphalium uliginosum, Juncus buffonius, Matricaria discoidea, Medicago lupulina, Plantago major, Poa annua, Polygonum aviculare, Ranunculus repens, Tanacetum vulgare, Trifolium repens, Vicia tetrasperma*

47C (herb cover 85%): *Agrostis stolonifera, Artemisia vulgaris, Cerastium holosteoides, Elytrigia repens, Galeopsis bifida, Gnaphalium uliginosum, Juncus buffonius, Matricaria discoidea, Persicaria lapathifolia, Phleum pratense, Plantago major, Poa annua, Polygonum aviculare, Prunella vulgaris, Ranunculus repens, Rumex crispus, Taraxacum* sect. *Ruderalia, Trifolium pratense, T. repens, Veronica arvensis*

47D (herb cover 95%): *Agrostis stolonifera, Arctium tomentosum, Artemisia vulgaris, Atriplex patula, Capsella bursa-pastoris, Cerastium holosteoides, Elytrigia repens, Gnaphalium uliginosum, Matricaria discoidea, Medicago lupulina, Plantago major, Poa annua, Polygonum aviculare, Ranunculus repens, Rorippa palustris, Stellaria graminea, Trifolium hybridum*
47E (herb cover 65%): *Agrostis stolonifera, Arctium tomentosum, Epilobium ciliatum, Glyceria notana, Gnaphalium uliginosum, Juncus buffonius, Leontodon autumnalis, Matricaria discoidea, Medicago lupulina, Plantago major, Poa annua, Polygonum aviculare, Ranunculus repens, Rumex crispus, Sagina procumbens, Taraxacum* sect. *Ruderalia, Trifolium hybridum, T. repens, Tussilago farfara*
48A (herb cover 95%): *Atriplex patula, Capsella bursa-pastoris, Glyceria notana, Chenopodium album, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare, Thlaspi arvense, Trifolium repens*
48B (herb cover 70%): *Gnaphalium uliginosum, Chenopodium glaucum, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Trifolium repens, Vicia hirsuta*
48C (herb cover 100%): *Atriplex patula, Glyceria notana, Lolium perenne, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare, Trifolium repens*
48D (herb cover 100%): *Agrostis stolonifera, Atriplex patula, Capsella bursa-pastoris, Elytrigia repens, Matricaria discoidea, Phleum pratense, Plantago major, Poa annua, Polygonum aviculare, Stellaria graminea, Taraxacum* sect. *Ruderalia, Trifolium repens*
48E (herb cover 95%): *Agrostis stolonifera, Capsella bursa-pastoris, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare, Trifolium repens*
49A (herb cover 80%): *Agrostis stolonifera, Achillea millefolium, Cerastium arvense, Dactylis glomerata, Festuca rubra, Matricaria discoidea, Plantago lanceolata, P. major, Ranunculus repens, Tragopogon orientalis, Trifolium pratense, T. repens*
49B (herb cover 70%): *Agrostis stolonifera, Achillea millefolium, Cerastium arvense, Festuca rubra, Galium album* agg., *Plantago lanceolata, P. major, Trifolium repens*
49C (herb cover 85%): *Agrostis stolonifera, Achillea millefolium, Cerastium holosteoides, Dactylis glomerata, Festuca pratensis, F. rubra, Leontodon autumnalis, Plantago lanceolata, P. major, Polygonum aviculare, Sonchus arvensis, Taraxacum* sect. *Ruderalia, Tragopogon orientalis, Trifolium repens*
49D (herb cover 85%): *Agrostis stolonifera, Achillea millefolium, Dactylis glomerata, Festuca rubra, Leontodon autumnalis, Pimpinella saxifraga, Plantago lanceolata, P. major, Poa pratensis, Polygonum aviculare, Trifolium repens*
49E (herb cover 95%): *Agrostis capillaris, Alchemilla* sp., *Avenula pubescens, Dactylis glomerata, Festuca pratensis, F. rubra, Galium album* agg., *Plantago lanceolata, P. major, Poa pratensis, Ranunculus repens, Rosa canina, Taraxacum* sect. *Ruderalia, Tragopogon orientalis, Trifolium repens, Veronica chamaedrys*
50A (herb cover 35%): *Agrostis stolonifera, Plantago major, Poa annua, Spergularia rubra*
50B (herb cover 70%): *Agrostis stolonifera, Dactylis glomerata, Geranium pussilum, Matricaria discoidea, Medicago lupulina, Melilotus officinalis, Plantago lanceolata, P. major, Poa annua, P. compressa, Polygonum aviculare, Potentilla anserina, Puccinelia distans, Sonchus oleraceus, Taraxacum* sect. *Ruderalia, Trifolium hybridum*
50C (herb cover 40%): *Agrostis stolonifera, Artemisia vulgaris, Cirsium arvense, Leontodon autumnalis, Matricaria discoidea, Melilotus officinalis, Plantago major, Poa annua, Polygonum aviculare, Puccinelia distans, Sonchus asper, Trifolium dubium, T. hybridum*
50D (herb cover 90%): *Agrostis stolonifera, Anagallis arvensis, Artemisia vulgaris, Capsella bursa-pastoris, Cerastium holosteoides, Cirsium arvense, Daucus carota, Epilobium tetragonum, Holcus lanatus, Hypericum perforatum, Chenopodium album, Matricaria discoidea, Melilotus officinalis, Plantago lanceolata, P. major, Poa annua, P. palustris, Tanacetum vulgare, Trifolium hybridum, T. pratense, T. repens, Vicia tetrasperma*
50E (herb cover 75%): *Agrostis stolonifera, Achillea millefolium, Anagallis arvensis, Capsella bursa-pastoris, Dactylis glomerata, Daucus carota, Lolium perenne, Matricaria discoidea, Melilotus officinalis, Plantago lanceolata, P. major, Poa annua, P. compressa, Polygonum aviculare, Puccinelia distans, Veronica arvensis*
51A (herb cover 80%): *Anagallis arvensis, Atriplex prostrata, Avena sativa, Capsella bursa-pastoris, Cirsium arvense, Echinochloa crus-galli, Elytrigia repens, Fallopia convolvulus, Hordeum vulgare, Chenopodium album, Lamium purpureum, Lolium perenne, Plantago major, Poa annua, P. trivialis, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Trifolium repens, Vicia hirsuta, Viola arvensis*
51B (herb cover 80%): *Elytrigia repens, Fallopia convolvulus, Hordeum vulgare, Chenopodium album, Lolium perenne, Plantago major, Poa annua, P. trivialis, Polygonum aviculare, Stellaria media, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Veronica persica, Vicia hirsuta*
51C (herb cover 85%): *Anagallis arvensis, Avena sativa, Elytrigia repens, Epilobium tetragonum, Fallopia convolvulus, Hordeum vulgare, Chenopodium album, Lolium perenne, Plantago major, Poa annua, Polygonum aviculare, Spergula arvensis, Stellaria media, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Vicia hirsuta, Viola arvensis*
51D (herb cover 75%): *Anagallis arvensis, Artemisia vulgaris, Avena fatua, A. sativa, Capsella bursa-pastoris, Cirsium arvense, Elytrigia repens, Fallopia convolvulus, Hordeum vulgare, Chenopodium album, Lamium purpureum, Matricaria recutita, Myosotis arvensis, Plantago major, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Vicia tetrasperma, Viola arvensis*
51E (herb cover 85%): *Agrostis gigantea, Anagallis arvensis, Artemisia vulgaris, Avena sativa, Cirsium arvense, Elytrigia repens, Fallopia convolvulus, Hordeum vulgare, Chenopodium album, Lolium perenne, Plantago major, Polygonum aviculare, Sonchus arvensis, Spergula arvensis, Taraxacum* sect. *Ruderalia, Veronica persica, Vicia hirsuta, V. tetrasperma, Viola arvensis*
52A (herb cover 65%): *Cirsium arvense, Galium aparine, Hordeum vulgare, Lolium perenne, Poa annua, P. trivialis, Taraxacum* sect. *Ruderalia, Veronica arvensis, V. persica*
52B (herb cover 90%): *Avena fatua, Capsella bursa-pastoris, Cirsium arvense, Elytrigia repens, Galium aparine, Lolium perenne, Taraxacum* sect. *Ruderalia, Triticum aestivum, Veronica arvensis, V. persica*

52C (herb cover 70%): *Anagallis arvensis, Avena fatua, Cirsium arvense, Galium aparine, Hordeum vulgare, Lolium perenne, Myosotis arvensis, Poa annua, P. trivialis, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Veronica arvensis*
52D (herb cover 95%): *Anagallis arvensis, Apera spica-venti, Avena fatua, Galium aparine, Lolium perenne, Polygonum aviculare, Veronica arvensis*
52E (herb cover 75%): *Apera spica-venti, Avena fatua, Capsella bursa-pastoris, Elytrigia repens, Galium aparine, Hordeum vulgare, Lolium perenne, Poa annua, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Veronica arvensis, V. persica, Viola arvensis*
53A (herb cover 70%): *Aethusa cynapium, Agrostis stolonifera, Anagallis arvensis, Artemisia vulgaris, Elytrigia repens, Galeopsis tetrahit, Hypericum perforatum, Lolium perenne, Poa annua, Triticum aestivum, Veronica persica*
53B (herb cover 50%): *Aethusa cynapium, Agrostis stolonifera, Anagallis arvensis, Artemisia vulgaris, Capsella bursa-pastoris, Elytrigia repens, Euphorbia helioscopia, Galeopsis tetrahit, Galium aparine, Lolium perenne, Myosotis arvensis, Poa annua, Stellaria media, Triticum aestivum, Veronica arvensis, V. persica*
53C (herb cover 40%): *Acer pseudoplatanus, Aethusa cynapium, Agrostis stolonifera, Anagallis arvensis, Apera spica-venti, Artemisia vulgaris, Galeopsis tetrahit, Lolium perenne, Plantago major, Poa annua, Polygonum aviculare, Triticum aestivum, Veronica arvensis, V. persica*
53D (herb cover 70%): *Anagallis arvensis, Apera spica-venti, Artemisia vulgaris, Elytrigia repens, Fumaria officinalis, Galeopsis tetrahit, Chenopodium album, Lolium perenne, Matricaria discoidea, Poa annua, Triticum aestivum, Veronica arvensis, V. persica*
53E (herb cover 85%): *Agrostis stolonifera, Elytrigia repens, Lolium perenne, Plantago major, Triticum aestivum, Veronica arvensis, V. persica*
54A (herb cover 40%): *Echinochloa crus-galli, Chenopodium album, Polygonum aviculare, Solanum nigrum, Taraxacum* sect. *Ruderalia, Viola arvensis*
54B (herb cover 50%): *Echinochloa crus-galli, Chenopodium album, Plantago major, Poa annua, Polygonum aviculare, Sinapis alba, Solanum nigrum, Viola arvensis*
54C (herb cover 20%): *Echinochloa crus-galli, Polygonum aviculare, Spergularia rubra, Viola arvensis*
54D (herb cover 40%): *Echinochloa crus-galli, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Viola arvensis*
54E (herb cover 95%): *Chenopodium album, Lolium perenne, Plantago major, Poa annua, Polygonum aviculare, Taraxacum* sect. *Ruderalia*
55A (herb cover 60%): *Echinochloa crus-galli, Elytrigia repens, Persicaria lapathifolia, Plantago major, Poa annua, Polygonum aviculare*
55B (herb cover 98%): *Chenopodium album, Plantago major, Polygonum aviculare, Raphanus raphanistrum, Viola arvensis*
55C (herb cover 80%): *Euphorbia helioscopia, Fallopia convolvulus, Chenopodium album, Papaver somniferum, Plantago major, Poa annua, Polygonum aviculare, Raphanus raphanistrum, Viola arvensis*
55D (herb cover 85%): *Agrostis stolonifera, Avena fatua, Capsella bursa-pastoris, Cirsium arvense, Euphorbia helioscopia, Fallopia convolvulus, Geranium pusillum, Chenopodium album, Lolium perenne, Polygonum aviculare*
55E (herb cover 50%): *Bromus hordeaceus, Fallopia convolvulus, Papaver somniferum, Poa annua, Polygonum aviculare*
56A (herb cover 80%): *Elytrigia repens, Fallopia convolvulus, Chenopodium album, Polygonum aviculare, Triticum aestivum, Viola arvensis*
56B (herb cover 60%): *Capsella bursa-pastoris, Elytrigia repens, Fallopia convolvulus, Chenopodium album, Lolium perenne, Polygonum aviculare, Triticum aestivum, Veronica persica*
56C (herb cover 90%): *Avena fatua, Cirsium arvense, Elytrigia repens, Medicago sativa, Papaver rhoeas, Poa annua, Veronica persica*
56D (herb cover 50%): *Descurainia sophia, Elytrigia repens, Chenopodium album, Lolium perenne, Polygonum aviculare*
56E (herb cover 70%): *Apera spica-venti, Capsella bursa-pastoris, Elytrigia repens, Fallopia convolvulus, Chenopodium album, Lolium perenne, Papaver rhoeas, Polygonum aviculare, Triticum aestivum*
57A (herb cover 70%): *Anagallis arvensis, Elytrigia repens, Fallopia convolvulus, Hypericum perforatum, Chenopodium album, Matricaria recutita, Plantago major, Poa annua, Polygonum aviculare, Raphanus raphanistrum, Silene latifolia, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Trifolium arvense*
57B (herb cover 50%): *Anagallis arvensis, Brassica napus, Matricaria recutita, Plantago major, Polygonum aviculare, Raphanus raphanistrum, Silene latifolia, Viola arvensis*
57C (herb cover 80%): *Anagallis arvensis, Brassica napus, Descurainia sophia, Elymus caninus, Polygonum aviculare, Raphanus raphanistrum, Silene latifolia, Viola arvensis*
57D (herb cover 80%): *Brassica napus, Capsella bursa-pastoris, Polygonum aviculare, Raphanus raphanistrum, Viola arvensis*
57E (herb cover 90%): *Brassica napus, Centauera cyanus, Elymus caninus, Geranium pusillum, Hypericum perforatum, Myosotis arvensis, Polygonum aviculare, Potentilla argentea, Silene latifolia, Viola arvensis*
58A (herb cover 95%): *Fallopia convolvulus, Chenopodium album, Papaver rhoeas, Poa annua, Polygonum aviculare, Viola arvensis*
58B (herb cover 80%): *Fallopia convolvulus, Chenopodium album, Lolium perenne, Myosotis arvensis, Papaver rhoeas, Poa annua, Polygonum aviculare, Triticum aestivum, Viola arvensis*
58C (herb cover 95%): *Chenopodium album, Lolium perenne, Myosotis arvensis, Poa annua, Polygonum aviculare, Triticum aestivum*
58D (herb cover 85%): *Anthriscus sylvestris, Apera spica-venti, Atriplex patula, Capsella bursa-pastoris, Elytrigia repens, Myosotis arvensis, Polygonum aviculare*
58E (herb cover 70%): *Capsella bursa-pastoris, Elytrigia repens, Fallopia convolvulus, Chenopodium album, Polygonum aviculare, Viola arvensis*

59A (herb cover 40%): *Agrostis stolonifera, Erodium cicutarium, Hordeum vulgare, Plantago major, Poa annua, Polygonum aviculare*
59B (herb cover 99%): *Agrostis stolonifera, Capsella bursa-pastoris, Cirsium arvense, Elytrigia repens, Fallopia convolvulus, Galium aparine, Geranium pusillum, Hordeum vulgare, Mentha arvensis, Myosotis arvensis, Urtica dioica, Vicia tetrasperma*
59C (herb cover 95%): *Apera spica-venti, Capsella bursa-pastoris, Cirsium arvense, Elytrigia repens, Fallopia convolvulus, Geranium pusillum, Hordeum vulgare, Mentha arvensis, Potentilla reptans, Urtica dioica, Vicia tetrasperma*
59D (herb cover 75%): *Agrostis stolonifera, Anthriscus sylvestris, Cirsium arvense, Erodium cicutarium, Euphorbia helioscopia, Hordeum vulgare, Chenopodium album, Matricaria discoidea, M. recutita, Mentha arvensis, Myosotis arvensis, Plantago major, Ranunculus repens, Thlaspi arvense, Trifolium repens, Veronica arvensis, V. polita*
59E (herb cover 95%): *Achillea millefolium, Elytrigia repens, Fallopia convolvulus, Hordeum vulgare, Chenopodium album, Persicaria lapathifolia, Pinus sylvestris, Plantago major, Polygonum aviculare, Potentilla reptans, Ranunculus repens, Spergula arvensis, Veronica arvensis, Viola arvensis*
60A (herb cover 70%): *Anagallis arvensis, Avena sativa, Fallopia convolvulus, Galium aparine, Glechoma hederacea, Chenopodium album, Lapsana communis, Papaver rhoeas, Taraxacum* sect. *Ruderalia, Trifolium repens, Triticum aestivum, Vicia hirsuta, Viola arvensis*
60B (herb cover 60%): *Agrostis stolonifera, Anagallis arvensis, Avena sativa, Centauera cyanus, Fallopia convolvulus, Galeopsis tetrahit, Geranium pusillum, Glechoma hederacea, Chenopodium album, Lapsana communis, Myosotis arvensis, Papaver rhoeas, Plantago lanceolata, Polygonum aviculare, Thlaspi arvense, Triticum aestivum, Vicia hirsuta, Viola arvensis*
60C (herb cover 50%): *Avena sativa, Centauera cyanus, Fallopia convolvulus, Lapsana communis, Papaver rhoeas, Setaria* sp., *Sonchus arvensis, Triticum aestivum, Veronica polita, Vicia hirsuta, Viola arvensis*
60D (herb cover 70%): *Avena sativa, Erodium cicutarium, Fallopia convolvulus, Galinsoga parviflora, Lapsana communis, Papaver rhoeas, Setaria* sp., *Sonchus arvensis, Triticum aestivum, Viola arvensis*
60E (herb cover 50%): *Anagallis arvensis, Avena sativa, Capsella bursa-pastoris, Cerastium arvense, Fallopia convolvulus, Festuca rubra, Hylotelephium maximum, Myosotis arvensis, Papaver rhoeas, Pillosella officinarum, Setaria* sp., *Triticum aestivum, Viola arvensis*
61A (herb cover 90%): *Brassica napus, Cirsium arvense, Consolida regalis, Fallopia convolvulus, Papaver rhoeas, Silene noctiflora, Vicia tetrasperma, Viola arvensis*
61B (herb cover 99%): *Brassica napus, Consolida regalis, Elytrigia repens, Fallopia convolvulus, Lactuca serriola, Papaver rhoeas, Silene noctiflora, Viola arvensis*
61C (herb cover 80%): *Brassica napus, Cirsium arvense, Elytrigia repens, Papaver rhoeas, Silene noctiflora, Viola arvensis*
61D (herb cover 80%): *Atriplex patula, Consolida regalis, Descurainia sophia, Fallopia convolvulus, Galium aparine, Lactuca serriola, Lolium perenne, Papaver rhoeas, Polygonum aviculare, Silene noctiflora, Triticum aestivum, Viola arvensis*
61E (herb cover 90%): *Anagallis arvensis, Brassica napus, Elytrigia repens, Papaver rhoeas, Polygonum aviculare, Triticum aestivum, Viola arvensis*
62A (herb cover 90%): *Brassica napus, Fallopia convolvulus, Chenopodium album, Poa annua, Polygonum aviculare*
62B (herb cover 60%): *Brassica napus, Fallopia convolvulus, Poa annua, Polygonum aviculare*
62C (herb cover 95%): *Brassica napus, Capsella bursa-pastoris, Descurainia sophia, Fallopia convolvulus, Polygonum aviculare, Viola arvensis*
62D (herb cover 97%): *Beta vulgaris, Brassica napus, Capsella bursa-pastoris, Descurainia sophia, Viola arvensis*
62E (herb cover 100%): *Capsella bursa-pastoris, Descurainia sophia, Fallopia convolvulus, Chenopodium album, Matricaria recutita, Polygonum aviculare, Thlaspi arvense, Viola arvensis*
63A (herb cover 50%): *Anagallis arvensis, Apera spica-venti, Bromus hordeaceus, Camelina microcarpa, Galium aparine, Hordeum vulgare, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare, Stellaria media, Triticum aestivum, Veronica persica*
63B (herb cover 60%): *Apera spica-venti, Echinochloa crus-galli, Geranium pusillum, Hordeum vulgare, Poa annua, Polygonum aviculare, Veronica persica*
63C (herb cover 75%): *Agrostis stolonifera, Apera spica-venti, Avena sativa, Echinochloa crus-galli, Hordeum vulgare, Plantago major, Polygonum aviculare, Stellaria media, Veronica persica*
63D (herb cover 60%): *Avena sativa, Capsella bursa-pastoris, Echinochloa crus-galli, Elytrigia repens, Galium aparine, Hordeum vulgare, Scleranthus annuus, Veronica persica*
63E (herb cover 78%): *Avena sativa, Echinochloa crus-galli, Hordeum vulgare, Lolium perenne, Polygonum aviculare, Veronica persica*
64A (herb cover 95%): *Brassica napus, Elytrigia repens, Fallopia convolvulus, Geranium pusillum, Hypericum perforatum, Lolium perenne, Lycopsis arvensis, Myosotis arvensis, Polygonum aviculare, Thlaspi arvense, Veronica arvensis, Viola arvensis*
64B (herb cover 90%): *Brassica napus, Elytrigia repens, Fallopia convolvulus, Geranium pusillum, Hypericum perforatum, Lactuca serriola, Lycopsis arvensis, Matricaria recutita, Polygonum aviculare, Silene latifolia, Thlaspi arvense, Viola arvensis*
64C (herb cover 70%): *Brassica napus, Geranium pusillum, Lycopsis arvensis, Polygonum aviculare, Thlaspi arvense, Viola arvensis*
64D (herb cover 80%): *Brassica napus, Hypericum perforatum, Lycopsis arvensis, Polygonum aviculare, Thlaspi arvense, Viola arvensis*
64E (herb cover 95%): *Brassica napus, Hypericum perforatum, Matricaria recutita, Polygonum aviculare, Viola arvensis*
65A (herb cover 95%): *Apera spica-venti, Brassica napus, Capsella bursa-pastoris, Cirsium arvense, Fallopia convolvulus, Geranium pusillum, Myosotis arvensis, Pastinaca sativa, Polygonum aviculare, Stellaria media, Triticum aestivum, Veronica arvensis, V. persica, Vicia cracca*

65B (herb cover 50%): *Apera spica-venti, Capsella bursa-pastoris, Geranium pusillum, Lamium purpureum, Myosotis arvensis, Pastinaca sativa, Poa annua, Polygonum aviculare, Rubus caesius, Stellaria media, Triticum aestivum, Veronica arvensis, V. persica*
65C (herb cover 95%): *Acer pseudoplatanus, Bromus hordeaceus, Capsella bursa-pastoris, Geranium pusillum, Lithospermum arvense, Myosotis arvensis, Polygonum aviculare, Raphanus raphanistrum, Rubus caesius, Stellaria media, Taraxacum* sect. *Ruderalia, Triticum aestivum, Veronica arvensis, Viola arvensis*
65D (herb cover 70%): *Brassica napus, Calystegia sepium, Geranium pusillum, Myosotis arvensis, Polygonum aviculare, Silene latifolia, Stellaria media, Triticum aestivum, Veronica arvensis, V. persica, Viola arvensis*
65E (herb cover 60%): *Apera spica-venti, Brassica napus, Geranium pusillum, Papaver rhoeas, Poa annua, Polygonum aviculare, Silene latifolia, Stellaria media, Triticum aestivum, Veronica arvensis, V. persica, Viola arvensis*
66A (herb cover 60%): *Anagallis arvensis, Avena fatua, Cirsium arvense, Echinochloa crus-galli, Elytrigia repens, Fallopia convolvulus, Poa annua, Polygonum aviculare, Trifolium repens, Triticum aestivum*
66B (herb cover 80%): *Anagallis arvensis, Avena fatua, Echinochloa crus-galli, Elytrigia repens, Fallopia convolvulus, Chenopodium album, Lolium perenne, Persicaria lapathifolia, Plantago major, Polygonum aviculare, Trifolium repens, Triticum aestivum*
66C (herb cover 82%): *Atriplex patula, Avena fatua, Chenopodium album, Polygonum aviculare, Triticum aestivum, Veronica persica*
66D (herb cover 90%): *Avena fatua, Capsella bursa-pastoris, Echinochloa crus-galli, Fallopia convolvulus, Poa annua, Polygonum aviculare, Triticum aestivum, Veronica persica*
66E (herb cover 80%): *Atriplex patula, Avena fatua, Fallopia convolvulus, Polygonum aviculare, Veronica persica, V. polita*
67A (herb cover 50%): *Apera spica-venti, Elytrigia repens, Lolium perenne, Plantago major, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Triticum aestivum, Veronica persica, V. polita, Viola arvensis*
67B (herb cover 45%): *Euphorbia helioscopia, Lolium perenne, Triticum aestivum, Veronica persica, V. polita*
67C (herb cover 50%): *Anagallis arvensis, Apera spica-venti, Lolium perenne, Poa annua, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Triticum aestivum, Veronica persica, V. polita*
67D (herb cover 70%): *Anagallis arvensis, Galium aparine, Lolium perenne, Poa annua, Polygonum aviculare, Triticum aestivum, Veronica persica, V. polita*
67E (herb cover 60%): *Apera spica-venti, Galium aparine, Chenopodium album, Lolium perenne, Plantago lanceolata, P. major, Poa annua, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Triticum aestivum, Viola arvensis*
68A (herb cover 99%): *Agrostis stolonifera, Artemisia vulgaris, Cirsium arvense, Elytrigia repens, Fallopia convolvulus, Chenopodium album, Lathyrus pratensis, Mentha arvensis, Myosotis arvensis, Plantago lanceolata, P. major, Poa trivialis, Potentilla anserina, Ranunculus repens, Silene noctiflora, Stellaria media, Thlaspi arvense, Trifolium repens, Triticum aestivum, Veronica arvensis, Vicia tetrasperma*
68B (herb cover 80%): *Agrostis stolonifera, Anagallis arvensis, Bistorta major, Capsella bursa-pastoris, Cirsium arvense, Euphorbia helioscopia, Fallopia convolvulus, Chenopodium album, Lathyrus pratensis, Mentha arvensis, Myosotis arvensis, Plantago major, Poa annua, Polygonum aviculare, Potentilla anserina, Ranunculus repens, Silene noctiflora, Thlaspi arvense, Triticum aestivum, Veronica arvensis*
68C (herb cover 90%): *Agrostis stolonifera, Artemisia vulgaris, Euphorbia helioscopia, Chenopodium album, Persicaria lapathifolia, Plantago major, Poa trivialis, Polygonum aviculare, Raphanus raphanistrum, Silene noctiflora, Stellaria media, Thlaspi arvense, Triticum aestivum, Veronica arvensis, Viola arvensis*
68D (herb cover 85%): *Agrostis stolonifera, Anagallis arvensis, Artemisia vulgaris, Euphorbia helioscopia, Hypericum perforatum, Chenopodium album, Mentha arvensis, Papaver rhoeas, Plantago major, Poa annua, Polygonum aviculare, Silene noctiflora, Stellaria media, Thlaspi arvense, Triticum aestivum, Veronica persica*
68E (herb cover 99%): *Agrostis stolonifera, Artemisia vulgaris, Elytrigia repens, Euphorbia helioscopia, Fallopia convolvulus, Galeopsis tetrahit, Chenopodium album, Mentha arvensis, Myosotis arvensis, Myosoton aquaticum, Phleum pratense, Plantago major, Poa trivialis, Polygonum aviculare, Ranunculus repens, Silene noctiflora, Sonchus arvensis, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Trifolium medium, T. repens, Triticum aestivum, Veronica arvensis, Vicia tetrasperma*
69A (herb cover 45%): *Capsella bursa-pastoris, Echinochloa crus-galli, Elytrigia repens, Chenopodium album, Polygonum aviculare, Veronica persica*
69B (herb cover 60%): *Artemisia vulgaris, Capsella bursa-pastoris, Echinochloa crus-galli, Elytrigia repens, Geranium pusillum, Chenopodium album, Stellaria media, Taraxacum* sect. *Ruderalia, Urtica dioica*
69C (herb cover 85%): *Artemisia vulgaris, Echinochloa crus-galli, Elytrigia repens, Chenopodium album, Myosotis arvensis, Poa annua, Polygonum aviculare, Stachys palustris, Viola arvensis*
69D (herb cover 80%): *Echinochloa crus-galli, Galium aparine, Chenopodium album, Persicaria amphibia, Polygonum aviculare, Stellaria media*
69E (herb cover 45%): *Artemisia vulgaris, Echinochloa crus-galli, Elytrigia repens, Chenopodium album, C. glaucum, C. polyspermum, Polygonum aviculare, Stellaria media*
70A (herb cover 80%): *Arrhenatherum elatius, Artemisia vulgaris, Cirsium arvense, Dactylis glomerata, Epilobium tetragonum, Taraxacum* sect. *Ruderalia, Trifolium repens, Vicia tetrasperma*
70B (herb cover 95%): *Anagallis arvensis, Artemisia vulgaris, Conyza canadensis, Dactylis glomerata, Epilobium tetragonum, Hypochaeris radiata, Taraxacum* sect. *Ruderalia, Trifolium repens*
70C (herb cover 80%): *Cerastium arvense, Conyza canadensis, Dactylis glomerata, Daucus carota, Epilobium tetragonum, Festuca rubra, Taraxacum* sect. *Ruderalia, Trifolium dubium, Veronica arvensis*
70D (herb cover 65%): *Achillea millefolium, Anagallis arvensis, Arenaria serpyllifolia, Cerastium arvense, Conyza canadensis, Dactylis glomerata, Epilobium tetragonum, Geranium pusillum, Hypochaeris radiata, Taraxacum* sect. *Ruderalia*

70E (herb cover 98%): *Anagallis arvensis, Artemisia vulgaris, Centaurea jacea, Cerastium arvense, Dactylis glomerata, Epilobium tetragonum, Geranium pusillum, Hypochaeris radiata, Taraxacum* sect. *Ruderalia, Veronica arvensis, Vicia tetrasperma, Viola arvensis*
71A (herb cover 90%): *Apera spica-venti, Plantago major, Triticum aestivum*
71B (herb cover 99%): *Agrostis stolonifera, Apera spica-venti, Elytrigia repens, Myosotis arvensis, Plantago major, Ranunculus repens, Taraxacum* sect. *Ruderalia, Trifolium hybridum, Triticum aestivum*
71C (herb cover 100%): *Agrostis stolonifera, Apera spica-venti, Elytrigia repens, Epilobium tetragonum, Myosotis arvensis, Plantago major, Ranunculus repens, Triticum aestivum*
71D (herb cover 80%): *Agrostis stolonifera, Apera spica-venti, Elytrigia repens, Matricaria recutita, Plantago major, Populus tremula, Triticum aestivum*
71E (herb cover 99%): *Agrostis stolonifera, Achillea millefolium, Apera spica-venti, Centauera cyanus, Elytrigia repens, Plantago major, Trifolium hybridum, Triticum aestivum, Vicia tetrasperma*
72A (herb cover 50%): *Anagallis arvensis, Echinochloa crus-galli, Lolium perenne, Papaver rhoeas, P. somniferum, Poa annua, Sonchus asper*
72B (herb cover 60%): *Bolboschoenus laticarpus, Fallopia convolvulus, Papaver rhoeas, P. somniferum, Poa annua*
72C (herb cover 80%): *Bolboschoenus laticarpus, Dactylis glomerata, Equisetum palustre, Lolium perenne, Papaver rhoeas, P. somniferum, Poa annua, Sonchus asper*
72D (herb cover 80%): *Bolboschoenus laticarpus, Echinochloa crus-galli, Fallopia convolvulus, Geranium pusillum, Lolium perenne, Papaver rhoeas, P. somniferum, Poa annua, Sonchus asper*
72E (herb cover 90%): *Bolboschoenus laticarpus, Echinochloa crus-galli, Fallopia convolvulus, Geranium dissectum, Lolium perenne, Papaver rhoeas, P. somniferum, Poa annua*
73A (herb cover 50%): *Avena fatua, Calystegia sepium, Echinochloa crus-galli, Elytrigia repens, Equisetum arvense, Euphorbia helioscopia, Fallopia convolvulus, Chenopodium album, Medicago lupulina, Persicaria lapathifolia, Silene noctiflora, Sonchus arvensis, Stellaria media, Tussilago farfara, Veronica persica, Vicia sativa, V. tetrasperma*
73B (herb cover 40%): *Euphorbia helioscopia, Fallopia convolvulus, Chenopodium album, Polygonum aviculare, Silene noctiflora, Sonchus arvensis, Thlaspi arvense, Veronica persica*
73C (herb cover 80%): *Avena sativa, Fallopia convolvulus, Fumaria officinalis, Galium aparine, Chenopodium album, Sonchus arvensis, Veronica persica*
73D (herb cover 95%): *Amaranthus retroflexus, Fallopia convolvulus, Galinsoga parviflora, Galium aparine, Chenopodium album, Silene noctiflora, Sinapis arvensis, Solanum tuberosum, Triticum aestivum*
73E (herb cover 88%): *Euphorbia helioscopia, Fallopia convolvulus, Fumaria officinalis, Galinsoga parviflora, Chenopodium album, Sinapis arvensis, Triticum aestivum, Vicia hirsuta, Viola arvensis*
74A (herb cover 70%): *Atriplex patula, Echinochloa crus-galli, Fallopia convolvulus, Sonchus asper, Thlaspi arvense, Viola arvensis*
74B (herb cover 60%): *Cirsium arvense, Elytrigia repens, Euphorbia helioscopia, Fallopia convolvulus, Lepidium ruderale, Papaver rhoeas, Sonchus asper, Thlaspi arvense, Viola arvensis*
74C (herb cover 55%): *Cirsium canum, Echinochloa crus-galli, Fallopia convolvulus, Sonchus oleraceus, Thlaspi arvense*
74D (herb cover 75%): *Atriplex patula, Fallopia convolvulus, Chenopodium album, Thlaspi arvense, Viola arvensis*
74E (herb cover 65%): *Euphorbia helioscopia, Chenopodium album, Papaver rhoeas, Thlaspi arvense, Viola arvensis*
75A (herb cover 80%): *Artemisia vulgaris, Bromus hordeaceus, B. sterilis, Capsella bursa-pastoris, Geranium pusillum, Chenopodium album, Plantago major, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Veronica persica, Viola arvensis*
75B (herb cover 85%): *Bromus hordeaceus, B. sterilis, Lolium perenne, Polygonum aviculare, Stellaria media, Taraxacum* sect. *Ruderalia, Veronica persica, Viola arvensis*
75C (herb cover 50%): *Bromus hordeaceus, B. sterilis, Lolium perenne, Plantago major, Poa annua, Veronica persica, Viola arvensis*
75D (herb cover 80%): *Acer pseudoplatanus, Bromus hordeaceus, Cirsium arvense, Plantago major, Poa annua, Polygonum aviculare, Puccinellia distans, Stellaria media, Veronica persica, Viola arvensis*
75E (herb cover 45%): *Capsella bursa-pastoris, Lolium perenne, Papaver rhoeas, Poa annua, Polygonum aviculare, Stellaria media, Viola arvensis*
76A (herb cover 99%): *Achillea millefolium, Arctium lappa, Artemisia vulgaris, Daucus carota, Galeopsis pubescens, Galeopsis tetrahit, Galium aparine, Chenopodium album, Lolium perenne, Medicago lupulina, M. sativa, Myosoton aquaticum, Plantago major, Poa annua, P. palustris, Polygonum aviculare, Potentilla anserina, Silene latifolia, Trifolium repens, Urtica dioica, Vicia cracca*
76B (herb cover 100%): *Cirsium arvense, Elytrigia repens, Chenopodium album, Melilotus albus, Myosoton aquaticum, Poa palustris, Polygonum aviculare, Rubus fruticosus* agg., *Urtica dioica, Vicia cracca*
76C (herb cover 99%): *Artemisia vulgaris, Atriplex sagittata, Cirsium arvense, Descurainia sophia, Elytrigia repens, Chenopodium album, Lamium purpureum, Medicago lupulina, Myosoton aquaticum, Plantago lanceolata, P. major, Poa palustris, Polygonum aviculare, Silene latifolia, Urtica dioica*
76D (herb cover 99%): *Achillea millefolium, Arctium lappa, Artemisia vulgaris, Cirsium arvense, Elytrigia repens, Chenopodium album, Lolium perenne, Malva neglecta, Melilotus albus, Plantago lanceolata, P. major, Polygonum aviculare, Potentilla anserina, Trifolium repens*
76E (herb cover 99%): *Arctium lappa, Artemisia vulgaris, Atriplex patula, Elytrigia repens, Melilotus albus, Phleum pratense, Plantago major, Poa annua, Polygonum aviculare, Rubus fruticosus* agg., *Triticum aestivum, Urtica dioica, Vicia cracca*
77A (herb cover 80%): *Agrostis gigantea, Galium aparine, Papaver rhoeas, Silene latifolia, Triticum aestivum*
77B (herb cover 72%): *Fumaria officinalis, Galeopsis tetrahit, Galium aparine, Polygonum aviculare, Silene latifolia, Triticum aestivum, Viola arvensis*

77C (herb cover 70%): *Agrostis gigantea, Galeopsis pubescens, Galeopsis tetrahit, Triticum aestivum, Viola arvensis*
77D (herb cover 85%): *Agrostis gigantea, Echinochloa crus-galli, Galium aparine, Persicaria lapathifolia, P. maculosa, Silene latifolia, Triticum aestivum, Viola arvensis*
77E (herb cover 95%): *Agrostis gigantea, Artemisia vulgaris, Daucus carota, Fallopia convolvulus, Galium aparine, Holcus mollis, Papaver rhoeas, Persicaria lapathifolia, Silene latifolia, Vicia tetrasperma, Viola arvensis*
78A (herb cover 99%): *Agrostis stolonifera, Atriplex patula, Calystegia sepium, Conyza canadensis, Dactylis glomerata, Lolium perenne, Matricaria discoidea, Plantago major, Poa annua, P. compressa*
78B (herb cover 90%): *Artemisia vulgaris, Atriplex patula, Calystegia sepium, Echinochloa crus-galli, Lolium perenne, Matricaria discoidea, Persicaria lapathifolia, Plantago major, Poa annua, Polygonum aviculare, Thlaspi arvense*
78C (herb cover 80%): *Agrostis stolonifera, Arctium lappa, Calystegia sepium, Dactylis glomerata, Chenopodium album, Lolium perenne, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare*
78D (herb cover 50%): *Agrostis stolonifera, Dactylis glomerata, Daucus carota, Epilobium tetragonum, Lathyrus pratensis, Lolium perenne, Matricaria discoidea, Plantago major, Poa annua, Ranunculus repens, Urtica dioica*
78E (herb cover 95%): *Agrostis stolonifera, Daucus carota, Matricaria discoidea, Plantago major, Polygonum aviculare, Populus tremula, Rubus fruticosus* agg., *Trifolium repens*
79A (herb cover 60%): *Chenopodium album, Papaver somniferum, Poa annua, Veronica persica*
79B (herb cover 85%): *Amaranthus retroflexus, Capsella bursa-pastoris, Chenopodium album, Papaver somniferum, Poa annua, Veronica persica*
79C (herb cover 99%): *Amaranthus retroflexus, Armoracia rusticana, Cirsium arvense, Elytrigia repens, Galinsoga quadriradiata, Galium aparine, Chenopodium album, Matricaria discoidea, M. recutita, Papaver somniferum, Poa annua, Polygonum aviculare, Stellaria media, Urtica dioica, Veronica arvensis*
79D (herb cover 90%): *Amaranthus retroflexus, Arctium lappa, Capsella bursa-pastoris, Holcus mollis, Chenopodium album, Papaver somniferum, Poa annua, Sonchus asper*
79E (herb cover 78%): *Galinsoga quadriradiata, Papaver somniferum, Salix fragilis, Stellaria media, Taraxacum* sect. *Ruderalia*
80A (herb cover 40%): *Apera spica-venti, Daucus carota, Elytrigia repens, Euphorbia helioscopia, Hypericum perforatum, Chenopodium album, Sonchus arvensis, Urtica dioica, Veronica persica*
80B (herb cover 77%): *Anagallis arvensis, Fallopia convolvulus, Polygonum aviculare, Sinapis alba, Thlaspi arvense, Veronica persica, Viola arvensis*
80C (herb cover 50%): *Brassica napus, Erodium cicutarium, Euphorbia helioscopia, Persicaria lapathifolia, Polygonum aviculare, Sinapis alba, Sonchus asper, Viola arvensis*
80D (herb cover 75%): *Erodium cicutarium, Euphorbia helioscopia, Lycopsis arvensis, Persicaria lapathifolia, Polygonum aviculare, Sinapis alba, Thlaspi arvense, Veronica persica, Viola arvensis*
80E (herb cover 80%): *Erodium cicutarium, Fallopia convolvulus, Geranium pusillum, Persicaria lapathifolia, Polygonum aviculare, Sinapis alba, Spergula arvensis, Viola arvensis*
81A (herb cover 80%): *Agrostis stolonifera, Anagallis arvensis, Echinochloa crus-galli, Elytrigia repens, Lolium perenne, Persicaria lapathifolia, Plantago major, Poa annua, Polygonum aviculare, Sinapis alba, Spergula arvensis, Triticum aestivum*
81B (herb cover 85%): *Anagallis arvensis, Capsella bursa-pastoris, Echinochloa crus-galli, Elytrigia repens, Geranium pusillum, Lolium perenne, Lycopsis arvensis, Plantago major, Poa annua, Polygonum aviculare, Sinapis alba, Spergula arvensis, Triticum aestivum, Veronica polita, Viola arvensis*
81C (herb cover 80%): *Capsella bursa-pastoris, Echinochloa crus-galli, Geranium pusillum, Lycopsis arvensis, Matricaria recutita, Papaver rhoeas, Plantago major, Poa annua, Polygonum aviculare, Sinapis alba, Spergula arvensis, Stellaria media, Taraxacum* sect. *Ruderalia, Veronica persica, Viola arvensis*
81D (herb cover 90%): *Anagallis arvensis, Capsella bursa-pastoris, Echinochloa crus-galli, Erodium cicutarium, Geranium pusillum, Matricaria recutita, Plantago major, Poa annua, Polygonum aviculare, Sinapis alba, Spergula arvensis, Thlaspi arvense, Veronica persica, Viola arvensis*
81E (herb cover 95%): *Anagallis arvensis, Echinochloa crus-galli, Elytrigia repens, Erodium cicutarium, Galeopsis tetrahit, Geranium pusillum, Lycopsis arvensis, Matricaria recutita, Persicaria lapathifolia, Poa annua, Polygonum aviculare, Sinapis alba, Spergula arvensis, Thlaspi arvense, Triticum aestivum, Veronica persica, Viola arvensis*
82A (herb cover 70%): *Amaranthus retroflexus, Cirsium arvense, Chenopodium album, Lolium perenne, Poa annua, Polygonum aviculare, Sonchus asper, Stellaria media, Taraxacum* sect. *Ruderalia*
82B (herb cover 75%): *Amaranthus retroflexus, Cirsium arvense, Echinochloa crus-galli, Galium aparine, Humulus lupulus, Chenopodium album, Poa annua, Stellaria media, Thlaspi arvense, Veronica persica*
82C (herb cover 90%): *Amaranthus retroflexus, Avena sativa, Holcus mollis, Chenopodium album, Lolium perenne, Stellaria media*
82D (herb cover 65%): *Amaranthus retroflexus, Cirsium arvense, Chenopodium album, Lolium perenne, Poa annua, Polygonum aviculare, Rumex crispus, Stellaria media*
82E (herb cover 55%): *Amaranthus retroflexus, Cirsium arvense, Echinochloa crus-galli, Chenopodium album*
83A (herb cover 70%): *Artemisia vulgaris, Echinochloa crus-galli, Elytrigia repens, Geranium dissectum, Chenopodium album, Plantago major, Polygonum aviculare*
83B (herb cover 20%): *Elytrigia repens, Chenopodium album, Plantago major, Zea mays*
83C (herb cover 95%): *Elytrigia repens, Euphorbia helioscopia, Fallopia convolvulus, Geranium pusillum, Chenopodium album, Poa annua, Viola arvensis, Zea mays*
83D (herb cover 65%): *Anagallis arvensis, Atriplex patula, Capsella bursa-pastoris, Echinochloa crus-galli, Plantago major, Poa annua, Polygonum aviculare*

83E (herb cover 99%): *Cirsium arvense, Echinochloa crus-galli, Lolium perenne, Matricaria discoidea, Polygonum aviculare*
84A (herb cover 75%): *Amaranthus retroflexus, Cerastium holosteoides, Convolvulus arvensis, Elytrigia repens, Euphorbia helioscopia, Chenopodium album, Lolium perenne, Taraxacum* sect. *Ruderalia*
84B (herb cover 60%): *Anthriscus sylvestris, Convolvulus arvensis, Dactylis glomerata, Elytrigia repens, Lolium perenne, Phleum pratense, Poa annua, Potentilla reptans, Taraxacum* sect. *Ruderalia, Tragopogon orientalis, Veronica arvensis*
84C (herb cover 50%): *Amaranthus retroflexus, Elytrigia repens, Lolium perenne, Phleum pratense, Stellaria media, Taraxacum* sect. *Ruderalia*
84D (herb cover 85%): *Festuca rubra, Galium aparine, Lolium perenne, Phleum pratense, Potentilla reptans, Taraxacum* sect. *Ruderalia, Veronica arvensis*
84E (herb cover 80%): *Anagallis arvensis, Anthriscus sylvestris, Convolvulus arvensis, Elytrigia repens, Lolium perenne, Phleum pratense, Poa annua, Polygonum aviculare, Potentilla reptans, Taraxacum* sect. *Ruderalia*
85A (herb cover 95%): *Arrhenatherum elatius, Cirsium arvense, Elytrigia repens, Festuca rubra, Taraxacum* sect. *Ruderalia, Trisetum flavescens*
85B (herb cover 90%): *Arrhenatherum elatius, Cirsium arvense, Dactylis glomerata, Elytrigia repens, Festuca rubra, Plantago lanceolata, Taraxacum* sect. *Ruderalia, Trisetum flavescens, Veronica arvensis*
85C (herb cover 85%): *Arrhenatherum elatius, Cirsium arvense, Dactylis glomerata, Elytrigia repens, Festuca rubra, Holcus mollis, Malva sylvestris, Poa pratensis*
85D (herb cover 90%): *Arrhenatherum elatius, Cirsium arvense, Dactylis glomerata, Elytrigia repens, Festuca rubra, Holcus mollis, Lathyrus pratensis, Setaria pumila, Trisetum flavescens*
85E (herb cover 85%): *Arrhenatherum elatius, Dactylis glomerata, Elytrigia repens, Festuca arundinacea, Picris hieracioides, Plantago major, Poa pratensis*
86A (herb cover 40%): *Anagallis arvensis, Anthemis arvensis, Apera spica-venti, Galeopsis tetrahit, Holcus mollis, Lapsana communis, Myosotis arvensis, Poa annua, Rumex acetosa, Trifolium repens, Triticum aestivum, Veronica arvensis, Vicia tetrasperma*
86B (herb cover 60%): *Anthemis arvensis, Apera spica-venti, Centaurea cyanus, Galeopsis tetrahit, Galium aparine, Lapsana communis, Triticum aestivum, Vicia sativa, V. tetrasperma, Viola arvensis*
86C (herb cover 50%): *Centaurea cyanus, Cirsium arvense, Triticum aestivum, Vicia sativa, Viola arvensis*
86D (herb cover 70%): *Anthemis arvensis, Apera spica-venti, Arabidopsis thaliana, Centaurea cyanus, Cirsium arvense, Galium aparine, Geranium pusillum, Holcus mollis, Lactuca serriola, Lolium perenne, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica arvensis, Vicia cracca, V. sativa, Viola arvensis*
86E (herb cover 60%): *Anagallis arvensis, Apera spica-venti, Capsella bursa-pastoris, Centaurea cyanus, Galium aparine, Lapsana communis, Myosotis arvensis, Triticum aestivum, Veronica arvensis, Vicia tetrasperma, Viola arvensis*
87A (herb cover 70%): *Agrostis capillaris, Achillea millefolium, Alopecurus pratensis, Anagallis arvensis, Anthemis arvensis, Anthriscus sylvestris, Arrhenathermum elatius, Artemsia vulgaris, Cirsium arvense, Fallopia convolvulus, Galeopsis tetrahit, Galium album* agg., *G. verum, Holcus lanatus, Lolium perenne, Myosotis arvensis, Plantago lanceolata, Rumex obtusifolius, Spergula arvensis, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica arvensis, V. chamaedrys, Vicia hirsuta, V. tetrasperma*
87B (herb cover 70%): *Agrostis capillaris, Achillea millefolium, Alopecurus pratensis, Anagallis arvensis, Capsella bursa-pastoris, Cirsium arvense, Galeopsis tetrahit, Juncus bufonius, Lapsana communis, Lolium perenne, Plantago lanceolata, P. major, Polygonum aviculare, Stellaria graminea, Trifolium pratense, Urtica dioica, Vicia hirsuta, V. sativa, V. tetrasperma, Viola arvensis*
87C (herb cover 100%): *Achillea millefolium, Anthriscus sylvestris, Apera spica-venti, Arrhenathermum elatius, Centaurea cyanus, Dactylis glomerata, Elytrigia repens, Galium aparine, Geranium pusillum, Holcus mollis, Lolium perenne, Poa pratensis, Stellaria graminea, Vicia hirsuta, V. sativa*
87D (herb cover 95%): *Achillea millefolium, Anthriscus sylvestris, Apera spica-venti, Arrhenathermum elatius, Centaurea cyanus, Elytrigia repens, Geranium pusillum, Holcus mollis, Lapsana communis, Stellaria graminea, Veronica arvensis, Vicia sativa*
87E (herb cover 60%): *Agrostis capillaris, Anagallis arvensis, Arabidopsis thaliana, Arrhenathermum elatius, Cirsium arvense, Fallopia convolvulus, Festuca rubra, Galeopsis tetrahit, Galium aparine, Holcus mollis, Lotus corniculatus, Myosotis arvensis, Poa pratensis, Ranunculus repens, Stellaria graminea, Trifolium repens, Urtica dioica, Verbascum nigrum, Vicia cracca, V. sativa*
88A (herb cover 100%): *Capsella bursa-pastoris, Elytrigia repens, Fallopia convolvulus, Galinsoga quadriradiata, Geranium pusillum, Chenopodium album, Lamium purpureum, Matricaria discoidea, Persicaria lapathifolia, Plantago major, Stellaria media, Taraxacum* sect. *Ruderalia, Trifolium pratense, Veronica persica*
88B (herb cover 95%): *Bromus hordeaceus, Capsella bursa-pastoris, Elytrigia repens, Galinsoga quadriradiata, Matricaria discoidea, Poa pratensis, Polygonum aviculare, Taraxacum* sect. *Ruderalia*
88C (herb cover 80%): *Artemsia vulgaris, Avena sativa, Elytrigia repens, Galinsoga quadriradiata, Galium aparine, Chenopodium album, Matricaria discoidea, Taraxacum* sect. *Ruderalia, Trifolium pratense, Triticum aestivum*
88D (herb cover 90%): *Artemsia vulgaris, Avena sativa, Elytrigia repens, Chenopodium album, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Trifolium pratense, Triticum aestivum*
88E (herb cover 95%): *Agrostis stolonifera, Achillea millefolium, Artemsia vulgaris, Convolvulus arvensis, Holcus mollis, Lolium perenne, Polygonum aviculare, Tanacetum vulgare, Taraxacum* sect. *Ruderalia, Trifolium pratense*
89A (herb cover 70%): *Echinochloa crus-galli, Chenopodium album, Taraxacum* sect. *Ruderalia, Viola arvensis*
89B (herb cover 70%): *Chenopodium album, Taraxacum* sect. *Ruderalia, Viola arvensis*
89C (herb cover 50%): *Alopecurus pratensis, Apera spica-venti, Capsella bursa-pastoris, Echinochloa crus-galli, Chenopodium album, Matricaria discoidea, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Viola arvensis*
89D (herb cover 70%): *Apera spica-venti, Capsella bursa-pastoris, Taraxacum* sect. *Ruderalia, Viola arvensis*

89E (herb cover 50%): *Fallopia convolvulus, Chenopodium album, C. polyspermum, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare, Thlaspi arvense*
90A (herb cover 50%): *Achillea millefolium, Apera spica-venti, Avena sativa, Capsella bursa-pastoris, Centaurea cyanus, Elytrigia repens, Chenopodium album, Myosotis arvensis, Polygonum aviculare, Trifolium reptans, Viola arvensis*
90B (herb cover 50%): *Apera spica-venti, Artemsia vulgaris, Avena sativa, Capsella bursa-pastoris, Centaurea cyanus, Elytrigia repens, Myosotis arvensis, Poa* sp., *Polygonum aviculare, Trifolium repens, Triticum aestivum*
90C (herb cover 50%): *Achillea millefolium, Apera spica-venti, Avena sativa, Capsella bursa-pastoris, Centaurea cyanus, Elytrigia repens, Fallopia convolvulus, Galium aparine, Geranium pusillum, Chenopodium album, Rumex acetosa, Triticum aestivum, Viola arvensis*
90D (herb cover 60%): *Apera spica-venti, Avena sativa, Centaurea cyanus, Fallopia convolvulus, Polygonum aviculare, Trifolium repens, Triticum aestivum*
90E (herb cover 50%): *Apera spica-venti, Avena sativa, Centaurea cyanus, Elytrigia repens, Fagopyrum esculentum, Galeopsis tetrahit, Galium aparine, Holcus mollis, Chenopodium album, Polygonum aviculare, Trifolium pratense, Triticum aestivum, Vicia hirsuta, Viola arvensis*
91A (herb cover 90%): *Aphanes arvensis, Capsella bursa-pastoris, Centaurea cyanus, Fallopia convolvulus, Galium aparine, Lamium purpureum, Polygonum aviculare, Stellaria media, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Veronica arvensis, Viola arvensis, Zea mays*
91B (herb cover 95%): *Centaurea cyanus, Fallopia convolvulus, Lamium purpureum, Myosotis arvensis, Phacelia tanacetifolia, Polygonum aviculare, Stellaria media, Taraxacum* sect. *Ruderalia, Zea mays*
91C (herb cover 50%): *Capsella bursa-pastoris, Echinochloa crus-galli, Fallopia convolvulus, Plantago major, Polygonum aviculare, Trifolium pratense, T. repens, Triticum aestivum, Viola arvensis, Zea mays*
91D (herb cover 60%): *Capsella bursa-pastoris, Centaurea cyanus, Elytrigia repens, Fallopia convolvulus, Galium aparine, Chenopodium album, Matricaria discoidea, Myosotis arvensis, Plantago major, Poa annua, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Trifolium repens*
91E (herb cover 95%): *Atriplex patula, Capsella bursa-pastoris, Centaurea cyanus, Fallopia convolvulus, Chenopodium album, Lamium purpureum, Matricaria discoidea, Polygonum aviculare, Stellaria media, Thlaspi arvense, Viola arvensis, Zea mays*
92A (herb cover 40%): *Echinochloa crus-galli, Elytrigia repens, Chenopodium album, Matricaria discoidea, Medicago sativa, Plantago major, Poa annua, P. palustris, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica persica*
92B (herb cover 60%): *Artemsia vulgaris, Capsella bursa-pastoris, Dactylis glomerata, Elytrigia repens, Chenopodium album, Matricaria discoidea, Medicago sativa, Phleum pratense, Plantago major, Poa annua, Polygonum aviculare, Taraxacum* sect. *Ruderalia*
92C (herb cover 80%): *Dactylis glomerata, Echinochloa crus-galli, Chenopodium album, Lolium perenne, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Trifolium pratense*
92D (herb cover 40%): *Apera spica-venti, Atriplex patula, Echinochloa crus-galli, Chenopodium album, Poa annua, P. palustris, Sambucus nigra, Taraxacum* sect. *Ruderalia, Urtica dioica*
92E (herb cover 50%): *Agrostis stolonifera, Artemsia vulgaris, Chenopodium album, Lolium perenne, Matricaria discoidea, Phleum pratense, Plantago major, Poa annua, Polygonum aviculare, Trifolium pratense*
93A (herb cover 95%): *Atriplex patula, Plantago lanceolata, Plantago major, Poa annua, Taraxacum* sect. *Ruderalia, Trifolium repens*
93B (herb cover 90%): *Atriplex patula, Capsella bursa-pastoris, Dactylis glomerata, Elytrigia repens, Erodium cicutarium, Geranium pusillum, Matricaria discoidea, Plantago major, Poa pratensis, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Trifolium pratense, Viola arvensis*
93C (herb cover 100%): *Lolium perenne, Plantago major, Poa pratensis, Polygonum aviculare, Puccinellia distans*
93D (herb cover 90%): *Dactylis glomerata, Lolium multiflorum, Lotus corniculatus, Poa pratensis, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Trifolium repens*
93E (herb cover 80%): *Apera spica-venti, Cerastium holosteoides, Dactylis glomerata, Plantago major, Poa pratensis, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Trifolium repens*
94A (herb cover 90%): *Agrostis stolonifera, Anagallis arvensis, Dactylis glomerata, Geranium dissectum, Juncus bufonius, Lolium perenne, Phleum pratense, Poa annua, Polygonum aviculare, Ranunculus repens, Rumex obtusifolius, Tanacetum vulgare*
94B (herb cover 90%): *Agrostis stolonifera, Elytrigia repens, Lolium perenne, Myosotis arvensis, Ranunculus repens, Rumex obtusifolius, Stellaria media, Taraxacum* sect. *Ruderalia*
94C (herb cover 90%): *Agrostis stolonifera, Achillea millefolium, Aphanes arvensis, Arrhenathermum elatius, Cerastium holosteoides, Dactylis glomerata, Geranium dissectum, Glechoma hederacea, Juncus bufonius, Lolium perenne, Myosotis arvensis, Phleum pratense, Plantago lanceolata, Rumex obtusifolius, Taraxacum* sect. *Ruderalia*
94D (herb cover 70%): *Agrostis stolonifera, Echinochloa crus-galli, Juncus bufonius, Leontodon autumnalis, Lolium perenne, Phleum pratense, Plantago major, Poa annua, Polygonum aviculare, Torilis japonica, Trifolium repens, Veronica arvensis*
94E (herb cover 30%): *Agrostis stolonifera, Geranium pusillum, Lolium perenne, Poa trivialis, Rumex obtusifolius*
95A (herb cover 70%): *Atriplex patula, Echinochloa crus-galli, Elytrigia repens, Galeopsis tetrahit, Chenopodium album, Lupinus polyphyllus, Persicaria hydropiper, Plantago uliginosa, Poa palustris, Polygonum aviculare*
95B (herb cover 70%): *Atriplex patula, Echinochloa crus-galli, Elytrigia repens, Galeopsis tetrahit, Gnaphalium uliginosum, Chenopodium album, Lupinus polyphyllus, Persicaria hydropiper, Poa annua, Taraxacum* sect. *Ruderalia, Trifolium hybridum*
95C (herb cover 80%): *Galinsoga quadriradiata, Chenopodium album, C. polyspermum, Lupinus polyphyllus, Poa annua, Taraxacum* sect. *Ruderalia*

95D (herb cover 70%): *Chenopodium album, Lupinus polyphyllus, Poa annua, Taraxacum* sect. *Ruderalia*
95E (herb cover 80%): *Elytrigia repens, Geranium pusillum, Knautia arvensis, Lupinus polyphyllus, Poa annua, Robinia pseudacacia, Vicia villosa*
96A (herb cover 40%): *Amaranthus retroflexus, Cirsium arvense, Echinochloa crus-galli, Chenopodium album, Poa annua, Polygonum aviculare, Viola arvensis*
96B (herb cover 90%): *Alopecurus pratensis, Bidens frondosa, Carex hirta, Cirsium arvense, Euphorbia helioscopia, Geranium dissectum, Lamium purpureum, Myosotis arvensis, Ranunculus repens, Thlaspi arvense, Trifolium hybridum, Vicia tetrasperma, Zea mays*
96C (herb cover 30%): *Agrostis stolonifera, Apera spica-venti, Lolium perenne, Poa annua, P. trivialis, Polygonum aviculare*
96D (herb cover 80%): *Agrostis stolonifera, Arrhenathermum elatius, Echinochloa crus-galli, Elytrigia repens, Equisetum arvense, Fallopia convolvulus, Holcus mollis, Lathyrus pratensis, Lotus corniculatus, Myosotis arvensis, Myosurus minimus, Vicia hirsuta*
96E (herb cover 80%): *Brassica napus, Elytrigia repens, Galium aparine, Thlaspi arvense, Vicia hirsuta, Zea mays*
97A (herb cover 50%): *Agrostis stolonifera, Apera spica-venti, Cirsium arvense, Conyza canadensis, Echinochloa crus-galli, Equisetum arvense, Fallopia convolvulus, Chenopodium album, Juncus bufonius, Myosotis arvensis, Plantago uliginosa, Poa annua, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Viola arvensis*
97B (herb cover 50%): *Apera spica-venti, Cerastium holosteoides, Conyza canadensis, Echinochloa crus-galli, Epilobium tetragonum, Hypericum perforatum, Myosotis arvensis, Persicaria lapathifolia, Plantago uliginosa, Poa annua, Polygonum aviculare, Ranunculus repens, Taraxacum* sect. *Ruderalia, Viola arvensis*
97C (herb cover 60%): *Agrostis stolonifera, Apera spica-venti, Arrhenathermum elatius, Conyza canadensis, Elytrigia repens, Epilobium tetragonum, Lactuca serriola, Trifolium repens, Viola arvensis*
97D (herb cover 80%): *Anagallis arvensis, Echinochloa crus-galli, Elytrigia repens, Epilobium tetragonum, Euphorbia helioscopia, Galeopsis tetrahit, Chenopodium album, Lactuca serriola, Ranunculus repens, Stellaria media, Trifolium pratense*
97E (herb cover 20%): *Arenaria serpyllifolia, Capsella bursa-pastoris, Cirsium arvense, Conyza canadensis, Echinochloa crus-galli, Elytrigia repens, Lactuca serriola, Myosotis arvensis, Plantago uliginosa, Poa annua, Veronica arvensis, Viola arvensis*
98A (herb cover 15%): *Agrostis stolonifera, Apera spica-venti, Centaurea cyanus, Echinochloa crus-galli, Elytrigia repens, Chenopodium polyspermum, Poa annua, Polygonum aviculare, Rumex obtusifolius, Vicia lathyroides, Viola arvensis*
98B (herb cover 35%): *Carex hirta, Conyza canadensis, Echinochloa crus-galli, Glyceria declinata, Persicaria lapathifolia, Ranunculus repens, Rorippa palustris, Stellaria uliginosa, Trifolium hybridum, Urtica dioica, Vicia sativa*
98C (herb cover 50%): *Agrostis stolonifera, Conyza canadensis, Epilobium ciliatum, Fallopia convolvulus, Chenopodium album, Lysimachia vulgaris, Poa annua, Ranunculus flammula, Rumex maritimus, Rumex obtusifolius, Trifolium hybridum*
98D (herb cover 30%): *Agrostis stolonifera, Cirsium arvense, Conyza canadensis, Echinochloa crus-galli, Epilobium ciliatum, Galium uliginosum, Hypericum perforatum, Juncus compressus, Plantago uliginosa, Poa palustris, Polygonum aviculare, Ranunculus repens, Trifolium hybridum, Veronica arvensis, Vicia tetrasperma*
98E (herb cover 30%): *Agrostis canina, Alopecurus aequalis, Apera spica-venti, Conyza canadensis, Epilobium ciliatum, Glyceria declinata, Holcus lanatus, Juncus articulatus, J. bufonius, Lysimachia vulgaris, Persicaria maculosa, Plantago lanceolata, P. uliginosa, Poa annua, P. palustris, Ranunculus flammula, R. repens, Trifolium hybridum, Veronica serpyllifolia*
99A (herb cover 50%): *Alopecurus aequalis, Atriplex patula, Bolboschoenus yagara, Carex bohemica, Echinochloa crus-galli, Epilobium* sp., *Juncus articulatus, J. bufonius, Myosoton aquaticum, Oenanthe aquatica, Persicaria maculosa, Poa trivialis, Ranunculus sceleratus, Rumex maritimus, Trifolium repens*
99B (herb cover 30%): *Apera spica-venti, Carex bohemica, Echinochloa crus-galli, Persicaria maculosa, Poa trivialis, Trifolium reptans*
99C (herb cover 80%): *Bolboschoenus yagara, Carex bohemica, Epilobium* sp., *Juncus articulatus, Oenanthe aquatica, Persicaria hydropiper, P. lapathifolia, P. maculosa, Rorippa palustris, Trifolium pratense, T. repens*
99D (herb cover 100%): *Alopecurus aequalis, Carex bohemica, Juncus articulatus, Oenanthe aquatica, Persicaria maculosa, Phalaris arundinacea, Rumex maritimus, Trifolium repens*
99E (herb cover 40%): *Alopecurus aequalis, Carex bohemica, Juncus articulatus, J. bufonius, Oenanthe aquatica, Poa trivialis, Trifolium repens*
100A (herb cover 20%): *Arrhenathermum elatius, Leontodon autumnalis, Puccinellia distans, Rumex acetosa, Spergularia rubra*
100B (herb cover 70%): *Calamagrostis epigejos, Digitaria ischaemum, Juncus bufonius, Leontodon autumnalis, Puccinellia distans, Spergularia rubra*
100C (herb cover 20%): *Arrhenathermum elatius, Equisetum arvense, Holcus mollis, Chenopodium album, Spergularia rubra*
100D (herb cover 50%): *Achillea millefolium, Calamagrostis epigejos, Carex hirta, Centaurea jacea, Festuca rubra, Genista tinctoria, Holcus mollis, Poa* sp., *Taraxacum* sect. *Ruderalia*
100E (herb cover 30%): *Agrostis capillaris, Arrhenathermum elatius, Equisetum arvense, Festuca rubra, Holcus mollis, Quercus robur, Rumex acetosa, Spergularia rubra*
101A (herb cover 70%): *Alopecurus aequalis, Anagallis arvensis, Glyceria declinata, Juncus bufonius, Matricaria discoidea, Persicaria hydropiper, Poa annua, P. trivialis, Polygonum aviculare, Triticum aestivum*
101B (herb cover 80%): *Alopecurus aequalis, Fallopia convolvulus, Galeopsis tetrahit, Glyceria declinata, Gnaphalium uliginosum, Juncus bufonius, Matricaria discoidea, Persicaria hydropiper, Plantago uliginosa, Poa annua, Polygonum aviculare, Rorippa palustris, Spergularia rubra, Triticum aestivum*
101C (herb cover 95%): *Alopecurus aequalis, Galeopsis tetrahit, Glyceria declinata, Matricaria discoidea, Persicaria hydropiper, Poa annua, Polygonum aviculare, Spergularia rubra, Triticum aestivum, Vicia tetrasperma*

101D (herb cover 100%): *Centaurea cyanus, Elytrigia repens, Galeopsis tetrahit, Galium aparine, Myosotis arvensis, Stellaria graminea, Triticum aestivum, Vicia hirsuta*
101E (herb cover 90%): *Alopecurus aequalis, Galeopsis tetrahit, Juncus bufonius, Matricaria discoidea, Persicaria hydropiper, Plantago uliginosa, Poa annua, Polygonum aviculare, Triticum aestivum, Vicia sativa*
102A (herb cover 60%): *Agrostis capillaris, Achillea millefolium, Artemsia vulgaris, Capsella bursa-pastoris, Festuca arundinacea, F. rubra, Pimpinella saxifraga, Plantago major, Polygonum aviculare, Rumex acetosa, Veronica arvensis*
102B (herb cover 50%): *Arrhenathermum elatius, Capsella bursa-pastoris, Hypericum perforatum, Lolium perenne, Matricaria discoidea, Plantago major, Polygonum aviculare, Rumex acetosa*
102C (herb cover 40%): *Achillea millefolium, Apera spica-venti, Arenaria serpyllifolia, Artemsia vulgaris, Dactylis glomerata, Lolium perenne, Pimpinella saxifraga, Plantago major, Poa annua, Polygonum aviculare, Sclerantus annuus, Taraxacum* sect. *Ruderalia*
102D (herb cover 30%): *Arrhenathermum elatius, Lolium perenne, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare, Rumex acetosa, Taraxacum* sect. *Ruderalia, Trifolium repens*
102E (herb cover 80%): *Agrostis capillaris, Arrhenathermum elatius, Artemsia vulgaris, Lactuca serriola, Lolium perenne, Myosotis arvensis, Poa annua, Polygonum aviculare, Ranunculus repens, Rumex acetosa, Senecio viscosus, Taraxacum* sect. *Ruderalia, Trifolium repens, Viola arvensis*
103A (herb cover 90%): *Capsella bursa-pastoris, Geranium pusillum, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare, Trifolium pratense, Triticum aestivum, Veronica persica*
103B (herb cover 95%): *Capsella bursa-pastoris, Fallopia convolvulus, Matricaria discoidea, Poa annua, Trifolium pratense, Triticum aestivum, Veronica persica*
103C (herb cover 90%): *Anagallis arvensis, Arrhenathermum elatius, Carduus acanthoides, Dactylis glomerata, Elytrigia repens, Fallopia convolvulus, Galeopsis tetrahit, Galium aparine, Geranium pusillum, Hypericum perforatum, Chenopodium album, Matricaria discoidea, Myosotis arvensis, Poa annua, Rubus fruticosus* agg., *Stellaria media, Trifolium pratense, Triticum aestivum, Veronica persica, Vicia sativa*
103D (herb cover 50%): *Agrostis capillaris, Achillea millefolium, Anagallis arvensis, Anthemis arvensis, Arrhenathermum elatius, Dianthus deltoides, Elytrigia repens, Fallopia convolvulus, Festuca rubra, Galium aparine, Geranium pusillum, Hypericum perforatum, Myosotis arvensis, Pimpinella saxifraga, Rumex acetosa, Sclerantus annuus, Trifolium medium, T. pratense, Triticum aestivum, Veronica arvensis, V. persica, Vicia sativa*
103E (herb cover 30%): *Agrostis capillaris, Artemsia vulgaris, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare*
104A (herb cover 100%): *Brassica napus, Centaurea cyanus, Convolvulus arvensis, Elytrigia repens, Galium aparine, Geranium pusillum, Silene latifolia, Viola arvensis*
104B (herb cover 100%): *Arrhenathermum elatius, Atriplex patula, Brassica napus, Elytrigia repens, Galeopsis tetrahit, Galium aparine, Silene latifolia, Thlaspi arvense*
104C (herb cover 100%): *Brassica napus, Galium aparine, Geranium pusillum, Silene latifolia, Viola arvensis*
104D (herb cover 100%): *Brassica napus, Capsella bursa-pastoris, Erigeron annuus, Galium aparine, Persicaria hydropiper, Poa annua, P. trivialis, Rumex obtusifolius, Thlaspi arvense, Veronica persica, Vicia hirsuta, Viola arvensis*
104E (herb cover 80%): *Brassica napus, Elytrigia repens, Epilobium tetragonum, Poa trivialis, Rumex obtusifolius*
105A (herb cover 50%): *Silene latifolia, Taraxacum* sect. *Ruderalia, Trifolium pratense, Viola arvensis*
105B (herb cover 60%): *Geranium pusillum, Polygonum aviculare, Silene latifolia, Taraxacum* sect. *Ruderalia, Trifolium pratense, Veronica arvensis*
105C (herb cover 50%): *Agrostis capillaris, Dactylis glomerata, Geranium pusillum, Lolium perenne, Quercus robur, Trifolium pratense, Veronica arvensis, Viola arvensis*
105D (herb cover 40%): *Agrostis capillaris, Festuca rubra, Knautia arvensis, Silene latifolia, Trifolium pratense, Triticum aestivum*
105E (herb cover 40%): *Apera spica-venti, Arrhenathermum elatius, Carex hirta, Geranium pusillum, Lapsana communis, Trifolium pratense*
106A (herb cover 60%): *Artemsia vulgaris, Dactylis glomerata, Equisetum arvense, Galium album* agg., *Chenopodium album, Phleum pratense, Poa annua, P. pratensis, Polygonum aviculare, Potentilla anserina, Ranunculus repens, Urtica dioica, Viola arvensis*
106B (herb cover 50%): *Arrhenathermum elatius, Fallopia convolvulus, Galeopsis tetrahit, Phalaris arundinacea, Plantago major, Poa annua, Polygonum aviculare, Potentilla anserina, Ranunculus repens, Stellaria graminea, Urtica dioica, Vicia sativa*
106C (herb cover 30%): *Achillea millefolium, Arrhenathermum elatius, Artemsia vulgaris, Capsella bursa-pastoris, Elytrigia repens, Equisetum arvense, Lolium perenne, Polygonum aviculare*
106D (herb cover 30%): *Aegopodium podagraria, Anthriscus sylvestris, Arrhenathermum elatius, Cerastium holosteoides, Equisetum arvense, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare, Ranunculus repens, Urtica dioica, Vicia sativa*
106E (herb cover 40%): *Aegopodium podagraria, Arrhenathermum elatius, Brassica napus, Capsella bursa-pastoris, Cerastium holosteoides, Elytrigia repens, Matricaria discoidea, Papaver dubium, Plantago major, Poa annua, Polygonum aviculare, Ranunculus repens, Trifolium repens*
107A (herb cover 70%): *Achillea millefolium, Centaurea jacea, Elytrigia repens, Equisetum arvense, Phleum pratense, Plantago major, Polygonum aviculare, Potentilla argentea, Stellaria graminea*
107B (herb cover 70%): *Arrhenathermum elatius, Carex hirta, Cerastium holosteoides, Dactylis glomerata, Daucus carota, Elytrigia repens, Equisetum arvense, Lolium perenne, Phleum pratense, Plantago lanceolata, Polygonum aviculare, Vicia hirsuta*
107C (herb cover 60%): *Achillea millefolium, Artemsia vulgaris, Capsella bursa-pastoris, Geranium pusillum, Lolium perenne, Phleum pratense, Plantago lanceolata, P. major, Poa pratensis, Taraxacum* sect. *Ruderalia, Trifolium hybridum, T. repens*

107D (herb cover 50%): *Lolium perenne, Phleum pratense, Plantago lanceolata, P. major, Polygonum aviculare*
107E (herb cover 20%): *Agrostis stolonifera, Capsella bursa-pastoris, Dactylis glomerata, Elytrigia repens, Galium album* agg., *Lolium perenne, Phleum pratense, Plantago lanceolata, P. major, Trifolium hybridum, T. repens*
108A (herb cover 70%): *Agrostis stolonifera, Apera spica-venti, Carex bohemica, Cirsium arvense, Juncus bufonius, Lycopus europaeus, Myosoton aquaticum, Persicaria maculosa, Poa annua, P. palustris, Ranunculus sceleratus, Rorippa palustris, Rumex maritimus, R. obtusifolius, Trifolium hybridum, T. repens*
108B (herb cover 50%): *Agrostis stolonifera, Bidens radiata, Carex bohemica, Epilobium ciliatum, Holcus mollis, Juncus bufonius, Persicaria lapathifolia, Ranunculus flammula, R. sceleratus, Rumex maritimus, Trifolium hybridum*
108C (herb cover 40%): *Carex bohemica, Epilobium ciliatum, Chenopodium album, Juncus bufonius, Persicaria lapathifolia, Phalaris arundinacea, Ranunculus flammula, Rorippa palustris, Rumex maritimus, Trifolium hybridum, Typha latifolia, Vicia tetrasperma*
108D (herb cover 100%): *Alopecurus aequalis, Bidens radiata, Carex bohemica, Epilobium ciliatum, Holcus mollis, Persicaria lapathifolia, Poa palustris, Rumex maritimus, Trifolium hybridum*
108E (herb cover 80%): *Carex hirta, Echinochloa crus-galli, Chenopodium album, Persicaria maculosa, Phalaris arundinacea, Poa palustris, Rumex maritimus, R. obtusifolius, Trifolium repens*
109A (herb cover 80%): *Echinochloa crus-galli, Lolium multiflorum, Poa annua, Trifolium pratense, Veronica persica, Viola arvensis*
109B (herb cover 60%): *Lolium multiflorum, Matricaria discoidea, Poa annua*
109C (herb cover 70%): *Apera spica-venti, Lolium multiflorum, Poa annua, Veronica arvensis*
109D (herb cover 50%): *Atriplex patula, Lolium multiflorum, Poa annua, Polygonum aviculare*
109E (herb cover 70%): *Fallopia convolvulus, Glyceria declinata, Lolium multiflorum, Poa annua, Taraxacum* sect. *Ruderalia, Trifolium pratense*
110A (herb cover 70%): *Anthemis arvensis, Matricaria discoidea, Polygonum aviculare, Thlaspi arvense, Veronica persica, Vicia sativa, Viola arvensis*
110B (herb cover 40%): *Anagallis arvensis, Anthemis arvensis, Capsella bursa-pastoris, Elytrigia repens, Fallopia convolvulus, Galium album* agg., *Matricaria discoidea, Myosotis arvensis, Phleum pratense, Plantago uliginosa, Poa annua, P. pratensis, Veronica chamaedrys, V. persica, Viola arvensis*
110C (herb cover 50%): *Brassica napus, Matricaria discoidea, Phleum pratense, Plantago uliginosa, Poa annua, Thlaspi arvense*
110D (herb cover 80%): *Fallopia convolvulus, Matricaria discoidea, Persicaria lapathifolia, Plantago major, Poa annua, Polygonum aviculare, Thlaspi arvense, Veronica persica, Viola arvensis*
110E (herb cover 50%): *Arrhenathermum elatius, Capsella bursa-pastoris, Convolvulus arvensis, Lycopsis arvensis, Plantago lanceolata, Poa annua, Taraxacum* sect. *Ruderalia, Veronica persica, Vicia sativa, Viola arvensis*
111A (herb cover 70%): *Arabidopsis thaliana, Artemsia vulgaris, Capsella bursa-pastoris, Dactylis glomerata, Fallopia convolvulus, Galeopsis tetrahit, Galinsoga quadriradiata, Galium aparine, Geranium pusillum, Chenopodium album, Myosotis arvensis, Phleum pratense, Polygonum aviculare, Stellaria media, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Triticum aestivum, Veronica arvensis, V. persica, Viola arvensis*
111B (herb cover 60%): *Apera spica-venti, Artemsia vulgaris, Avena sativa, Capsella bursa-pastoris, Centaurea cyanus, Erodium cicutarium, Fallopia convolvulus, Galinsoga quadriradiata, Geranium pusillum, Chenopodium album, Lactuca serriola, Myosotis arvensis, Stellaria media, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Veronica arvensis, V. persica, Vicia hirsuta, Viola arvensis*
111C (herb cover 60%): *Achillea millefolium, Artemsia vulgaris, Capsella bursa-pastoris, Centaurea cyanus, Erodium cicutarium, Fallopia convolvulus, Galinsoga quadriradiata, Galium aparine, Geranium pusillum, Chenopodium album, Lactuca serriola, Myosotis arvensis, Stellaria media, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Veronica persica, Vicia hirsuta, Viola arvensis*
111D (herb cover 95%): *Apera spica-venti, Arrhenathermum elatius, Capsella bursa-pastoris, Dactylis glomerata, Elytrigia repens, Erodium cicutarium, Fallopia convolvulus, Galeopsis tetrahit, Galinsoga quadriradiata, Geranium pusillum, Chenopodium album, Lactuca serriola, Lolium perenne, Myosotis arvensis, Plantago major, Polygonum aviculare, Stellaria media, Thlaspi arvense, Triticum aestivum, Veronica arvensis, V. persica, Viola arvensis*
111E (herb cover 80%): *Achillea millefolium, Artemsia vulgaris, Avena sativa, Capsella bursa-pastoris, Centaurea cyanus, Cirsium arvense, Erodium cicutarium, Fallopia convolvulus, Galeopsis tetrahit, Galinsoga quadriradiata, Geranium pusillum, Glyceria declinata, Myosotis arvensis, Persicaria lapathifolia, Phleum pratense, Plantago lanceolata, Poa palustris, Rumex obtusifolius, Stellaria media, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Trifolium hybridum, Triticum aestivum, Veronica arvensis, V. persica, Vicia tetrasperma, Viola arvensis*
112A (herb cover 40%): *Apera spica-venti, Arrhenathermum elatius, Brassica napus, Calamagrostis epigejos, Echinochloa crus-galli, Elytrigia repens, Fallopia convolvulus, Poa annua, Trifolium pratense, Vicia tetrasperma, Viola arvensis*
112B (herb cover 30%): *Echinochloa crus-galli, Fallopia convolvulus, Juncus bulbosus, Persicaria lapathifolia, Polygonum aviculare, Trifolium pratense, Vicia tetrasperma*
112C (herb cover 50%): *Brassica napus, Elytrigia repens, Myosotis arvensis, Vicia tetrasperma, Viola arvensis*
112D (herb cover 50%): *Apera spica-venti, Echinochloa crus-galli, Plantago major, Poa annua, P. trivialis, Trifolium pratense, Vicia tetrasperma*
112E (herb cover 95%): *Apera spica-venti, Brassica napus, Elytrigia repens, Myosotis arvensis, Poa trivialis*
113A (herb cover 100%): *Artemsia vulgaris, Capsella bursa-pastoris, Centaurea cyanus, Elytrigia repens, Fallopia convolvulus, Galeopsis tetrahit, Chenopodium album, Polygonum aviculare, Stellaria media, Vicia sativa*
113B (herb cover 90%): *Geranium pusillum, Holcus mollis, Chenopodium album, Lolium perenne, Matricaria discoidea, Poa annua, Rumex acetosa, Stellaria media, Viola arvensis, Zea mays*
113C (herb cover 90%): *Elytrigia repens, Lolium perenne, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare*

113D (herb cover 95%): *Centaurea cyanus, Geranium pusillum, Lolium perenne, Poa annua, Silene latifolia, Stellaria media, Triticum aestivum, Vicia hirsuta, Viola arvensis, Zea mays*

113E (herb cover 20%): *Lolium perenne, Zea mays*

114A (herb cover 60%): *Apera spica-venti, Artemsia vulgaris, Capsella bursa-pastoris, Centaurea cyanus, Elytrigia repens, Fallopia convolvulus, Geranium dissectum, G. pusillum, Lamium purpureum, Lapsana communis, Matricaria recutita, Myosotis arvensis, Thlaspi arvense, Veronica persica, Vicia hirsuta, Viola arvensis*

114B (herb cover 60%): *Capsella bursa-pastoris, Centaurea cyanus, Dactylis glomerata, Elytrigia repens, Fallopia convolvulus, Geranium pusillum, Chenopodium album, Matricaria recutita, Myosotis arvensis, Silene latifolia, Thlaspi arvense, Vicia hirsuta, Viola arvensis*

114C (herb cover 70%): *Capsella bursa-pastoris, Centaurea cyanus, Elytrigia repens, Fallopia convolvulus, Geranium pusillum, Hypericum perforatum, Chenopodium album, Papaver somniferum, Viola arvensis*

114D (herb cover 90%): *Capsella bursa-pastoris, Centaurea cyanus, Elytrigia repens, Fallopia convolvulus, Geranium pusillum, Papaver somniferum, Silene latifolia, Thlaspi arvense, Veronica persica, Vicia hirsuta*

114E (herb cover 80%): *Arrhenathermum elatius, Elytrigia repens, Geranium pusillum, Lolium perenne, Papaver somniferum, Poa annua, Viola arvensis*

115A (herb cover 90%): *Echinochloa crus-galli, Fallopia convolvulus, Galium aparine, Polygonum aviculare, Solanum tuberosum*

115B (herb cover 60%): *Echinochloa crus-galli, Elytrigia repens, Fallopia convolvulus, Solanum tuberosum, Viola arvensis*

115C (herb cover 70%): *Centaurea cyanus, Echinochloa crus-galli, Fallopia convolvulus, Galinsoga quadriradiata, Lycopsis arvensis, Polygonum aviculare, Solanum tuberosum*

115D (herb cover 95%): *Avena sativa, Capsella bursa-pastoris, Echinochloa crus-galli, Erodium cicutarium, Galinsoga quadriradiata, Holcus mollis, Chenopodium album, Persicaria lapathifolia, Poa annua, Polygonum aviculare, Solanum tuberosum, Spergularia rubra, Stellaria media, Taraxacum* sect. *Ruderalia*

115E (herb cover 50%): *Capsella bursa-pastoris, Echinochloa crus-galli, Elytrigia repens, Fallopia convolvulus, Galinsoga quadriradiata, Chenopodium album, Matricaria discoidea, Persicaria lapathifolia, Poa annua, Polygonum aviculare, Solanum tuberosum, Spergularia rubra, Stellaria media, Thlaspi arvense*

116A (herb cover 40%): *Anagallis arvensis, Capsella bursa-pastoris, Echinochloa crus-galli, Elytrigia repens, Fallopia convolvulus, Poa annua, Viola arvensis*

116B (herb cover 60%): *Capsella bursa-pastoris, Echinochloa crus-galli, Elytrigia repens, Fallopia convolvulus, Plantago uliginosa, Polygonum aviculare, Viola arvensis*

116C (herb cover 70%): *Elytrigia repens, Fallopia convolvulus, Lamium purpureum, Persicaria maculosa, Plantago uliginosa, Poa annua, Polygonum aviculare, Stellaria media, Taraxacum* sect. *Ruderalia, Viola arvensis*

116D (herb cover 50%): *Agrostis stolonifera, Achillea millefolium, Anthemis arvensis, Apera spica-venti, Daucus carota, Elytrigia repens, Fallopia convolvulus, Geranium pusillum, Lolium perenne, Phleum pratense, Poa annua, Polygonum aviculare, Sclerantus annuus, Stellaria graminea, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Triticum aestivum, Vicia hirsuta, Viola arvensis*

116E (herb cover 50%): *Apera spica-venti, Arabidopsis thaliana, Centaurea cyanus, Elytrigia repens, Fallopia convolvulus, Geranium dissectum, Lolium perenne, Myosotis arvensis, Phleum pratense, Polygonum aviculare, Triticum aestivum, Vicia tetrasperma*

117A (herb cover 60%): *Achillea millefolium, Anagallis arvensis, Echinochloa crus-galli, Elytrigia repens, Geranium dissectum, Lolium perenne, Plantago major, Potentilla anserina, Ranunculus repens, Spergularia rubra, Trifolium repens, Vicia cracca, V. tetrasperma, Viola arvensis*

117B (herb cover 90%): *Anagallis arvensis, Capsella bursa-pastoris, Carex hirta, Echinochloa crus-galli, Elytrigia repens, Matricaria discoidea, Plantago major, Polygonum aviculare, Ranunculus repens, Spergularia rubra, Taraxacum* sect. *Ruderalia, Trifolium repens*

117C (herb cover 95%): *Agrostis capillaris, Arrhenathermum elatius, Capsella bursa-pastoris, Cirsium arvense, Elytrigia repens, Glyceria declinata, Chenopodium album, Lamium purpureum, Matricaria discoidea, Persicaria lapathifolia, Plantago uliginosa, Poa annua, Ranunculus repens, Spergularia rubra, Stellaria media, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Veronica persica, Zea mays*

117D (herb cover 70%): *Agrostis stolonifera, Centaurea cyanus, Elytrigia repens, Fallopia convolvulus, Galium aparine, Geranium dissectum, Lamium purpureum, Lapsana communis, Matricaria discoidea, Persicaria maculosa, Taraxacum* sect. *Ruderalia, Thlaspi arvense, Veronica persica, Viola arvensis, Zea mays*

117E (herb cover 80%): *Agrostis stolonifera, Echinochloa crus-galli, Glyceria declinata, Matricaria discoidea, Persicaria hydropiper, P. lapathifolia, Poa annua, Vicia tetrasperma, Zea mays*

118A (herb cover 90%): *Elytrigia repens, Epilobium ciliatum, Galeopsis tetrahit, Rumex maritimus, R. obtusifolius, Urtica dioica*

118B (herb cover 40%): *Calamagrostis arundinacea, Carex hirta, Elytrigia repens, Lapsana communis, Urtica dioica*

118C (herb cover 95%): *Elytrigia repens, Epilobium ciliatum, Persicaria hydropiper, Poa palustris, Rumex maritimus, R. obtusifolius, Urtica dioica*

118D (herb cover 60%): *Alnus glutiosa, Elytrigia repens, Myosotis palustris, Rumex obtusifolius, Salix caprea, Urtica dioica*

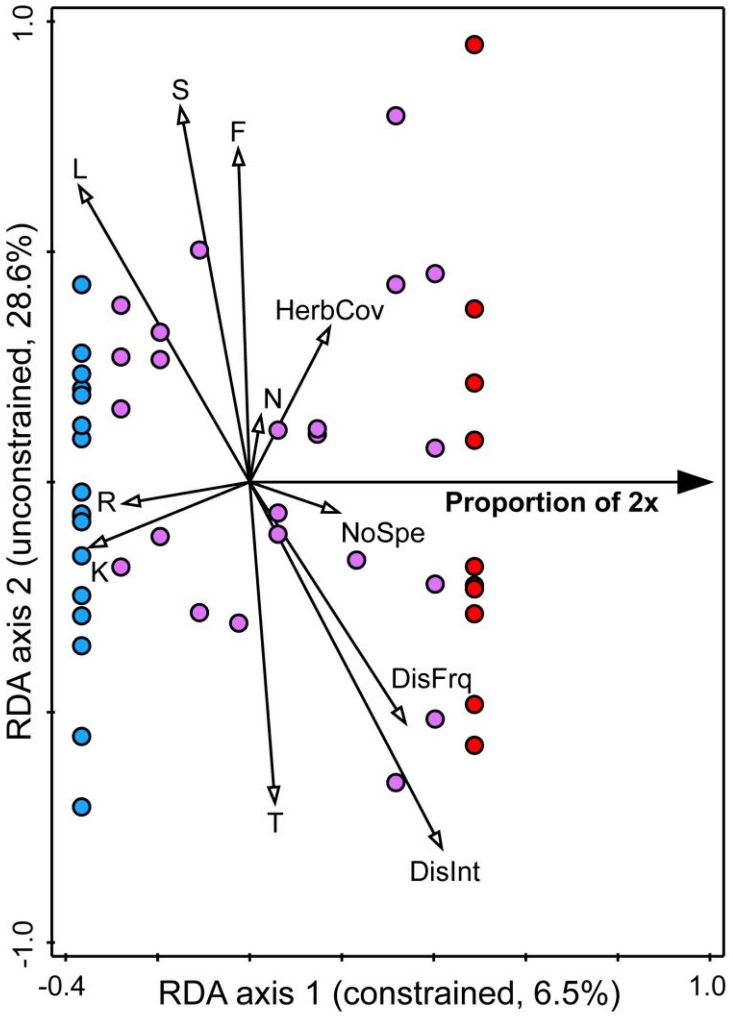
118E (herb cover 70%): *Alnus glutiosa, Elytrigia repens, Poa palustris, Rumex obtusifolius, Stellaria media, S. nemorum, Urtica dioica*

119A (herb cover 100%): *Alopecurus aequalis, Echinochloa crus-galli, Epilobium roseum, E. tetragonum, Persicaria hydropiper, P. lapathifolia, Phalaris arundinacea, Poa trivialis, Rorippa palustris, Trifolium hybridum*

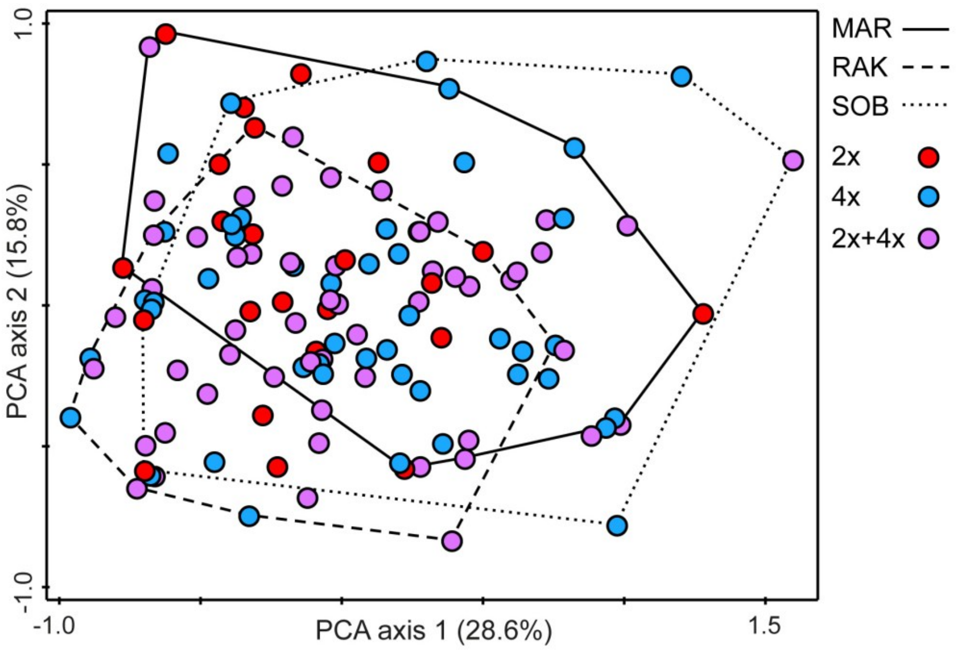
119B (herb cover 100%): *Apera spica-venti, Epilobium hirsutum, Glyceria maxima, Phalaris arundinacea, Poa trivialis, Rorippa palustris, Urtica dioica*

119C (herb cover 100%): *Atriplex patula, Epilobium hirsutum, E. tetragonum, Phalaris arundinacea, Rorippa palustris, Urtica dioica*

119D (herb cover 100%): *Alopecurus aequalis, Apera spica-venti, Epilobium tetragonum, Persicaria hydropiper, Phalaris arundinacea, Poa palustris, P. trivialis, Rorippa palustris, Urtica dioica*
119E (herb cover 100%): *Apera spica-venti, Epilobium tetragonum, Glyceria maxima, Phalaris arundinacea, Poa palustris, Rorippa palustris, Rumex maritimus, Urtica dioica*
120A (herb cover 80%): *Aegopodium podagraria, Calystegia sepium, Cirsium arvense, Elytrigia repens, Equisetum arvense, Festuca arundinacea, Chenopodium album, C. polyspermum, Matricaria discoidea, Poa annua, Taraxacum* sect. *Ruderalia, Trifolium repens, Urtica dioica*
120B (herb cover 90%): *Agrostis stolonifera, Anagallis arvensis, Calystegia sepium, Dactylis glomerata, Equisetum arvense, Poa annua, Polygonum aviculare, Taraxacum* sect. *Ruderalia, Trifolium repens, Urtica dioica*
120C (herb cover 80%): *Aegopodium podagraria, Agrostis stolonifera, Ballota nigra, Calystegia sepium, Cerastium holosteoides, Elytrigia repens, Hypericum perforatum, Chenopodium polyspermum, Lolium perenne, Matricaria discoidea, Plantago major, Poa annua, Polygonum aviculare, Ranunculus repens, Rumex obtusifolius, Taraxacum* sect. *Ruderalia, Trifolium repens*
120D (herb cover 90%): *Agrostis stolonifera, Achillea millefolium, Arctium lappa, Atriplex patula, Bellis perennis, Capsella bursa-pastoris, Elytrigia repens, Chenopodium polyspermum, Poa annua, Polygonum aviculare, Ranunculus repens, Rumex obtusifolius, Symphytum officinale, Taraxacum* sect. *Ruderalia, Urtica dioica, Veronica arvensis*
120E (herb cover 90%): *Aegopodium podagraria, Achillea millefolium, Arctium lappa, Ballota nigra, Capsella bursa-pastoris, Dactylis glomerata, Lolium multiflorum, L. perenne, Matricaria recutita, Plantago major, Poa annua, Taraxacum* sect. *Ruderalia, Trifolium repens, Veronica chamaedrys*
121A (herb cover 80%): *Apera spica-venti, Capsella bursa-pastoris, Echinochloa crus-galli, Juncus bufonius, Matricaria discoidea, Poa annua, Rorippa palustris, Triticum aestivum*
121B (herb cover 30%): *Capsella bursa-pastoris, Matricaria discoidea, Poa annua, Triticum aestivum*
121C (herb cover 90%): *Capsella bursa-pastoris, Cirsium arvense, Poa annua, Triticum aestivum*
121D (herb cover 75%): *Anthriscus sylvestris, Capsella bursa-pastoris, Echinochloa crus-galli, Juncus bufonius, Matricaria discoidea, Persicaria hydropiper, Poa annua, Triticum aestivum*
121E (herb cover 40%): *Echinochloa crus-galli, Juncus bufonius, Matricaria discoidea, Persicaria hydropiper, Poa annua*
122A (herb cover 90%): *Apera spica-venti, Conyza canadensis, Epilobium ciliatum, Lactuca serriola, Poa annua, Trifolium hybridum, Viola arvensis*
122B (herb cover 80%): *Apera spica-venti, Conyza canadensis, Epilobium ciliatum, Hypericum maculatum, Plantago major, Populus × canadensis, Stachys palustris, Taraxacum* sect. *Ruderalia*
122C (herb cover 70%): *Apera spica-venti, Betula pendula, Capsella bursa-pastoris, Conyza canadensis, Epilobium ciliatum, Lactuca serriola, Myosotis arvensis, Poa trivialis, Taraxacum* sect. *Ruderalia*
122D (herb cover 70%): *Agrostis capillaris, Apera spica-venti, Arrhenathermum elatius, Capsella bursa-pastoris, Conyza canadensis, Elytrigia repens, Holcus mollis, Rubus idaeus, Senecio sylvaticus, Vicia sativa*
122E (herb cover 50%): *Agrostis capillaris, Achillea millefolium, Capsella bursa-pastoris, Holcus mollis, Lactuca serriola, Sonchus asper, Trifolium repens, Vicia tetrasperma, Viola arvensis*
123A (herb cover 50%): *Agrostis stolonifera, Carex bohemica, Cirsium arvense, Dactylis glomerata, Eleocharis palustris, Juncus bulbosus, Litorella uniflora, Lycopus europaeus, Phalaris arundinacea, Plantago uliginosa, Ranunculus flammula, Rorippa palustris, Rumex obtusifolius, Triticum aestivum, Veronica scutellata*
123B (herb cover 50%): *Agrostis canina, Agrostis stolonifera, Bidens frondosa, B. tripartita, Carex bohemica, Glyceria notata, Juncus bulbosus, Lycopus europaeus, Phalaris arundinacea, Potentilla supina, Ranunculus flammula, Veronica scutellata*
123C (herb cover 80%): *Bidens frondosa, B. tripartita, Carex bohemica, Conyza canadensis, Epilobium* sp., *Glyceria notata, Gnaphalium uliginosum, Juncus bulbosus, Phalaris arundinacea, Pinus sylvestris, Plantago uliginosa, Ranunculus flammula, Veronica scutellata*
123D (herb cover 20%): *Agrostis capillaris, Alopecurus aequalis, Myosotis arvensis, Persicaria hydropiper, P. lapathifolia, Pinus sylvestris, Plantago uliginosa, Ranunculus flammula, Rorippa palustris, Veronica scutellata*
123E (herb cover 30%): *Alopecurus aequalis, Avena sativa, Bidens frondosa, B. tripartita, Carex bohemica, Echinochloa crus-galli, Lotus corniculatus, Ranunculus flammula, Trifolium repens, Triticum aestivum, Veronica scutellata*



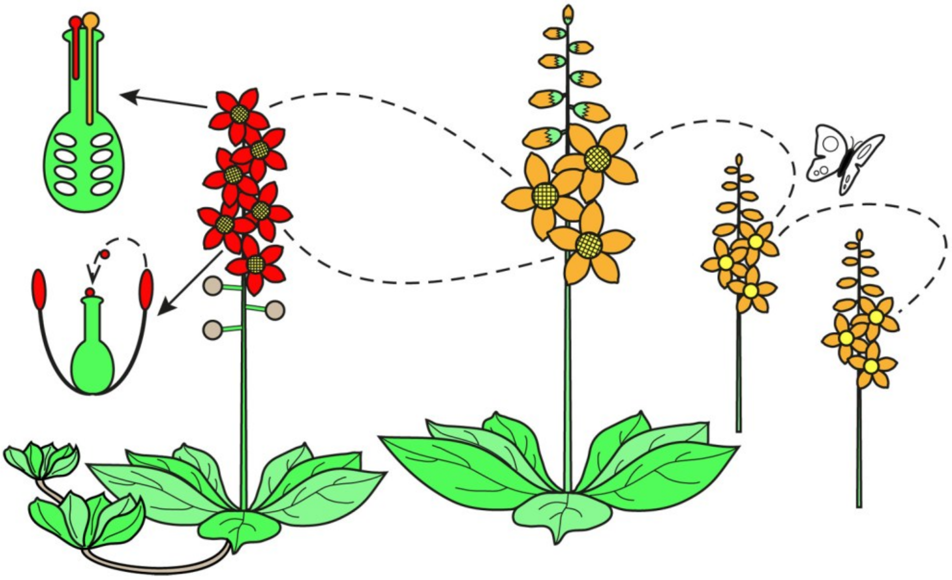
Appendix S4. A redundancy analysis of mean values of ecological variables derived from composition of vegetation in diploid (red), tetraploid (blue), and mixed-ploidy (violet) populations from the region MAR. Proportion of diploids in populations was used as a response variable. Eleven variables were used for ecological-niche reconstruction: number of species (NoSpe), relative cover of herb layer (HerbCov), index of disturbance intensity (DisInt), index of disturbance frequency (DisFrq), and Ellenberg indicator values for light (L), temperature (T), continentality (K), humidity (F), soil reaction (R), nutrients (N), and salinity (S).



Appendix S5. A principal component analysis of mean values of ecological variables derived from composition of vegetation in diploid (red), tetraploid (blue), and mixed-ploidy (violet) populations sampled in three different regions (MAR, RAK, SOB) of the contact zone. Lines delimit the degree of overlap in environmental conditions among the study regions.

PAPER V

Mixed-ploidy species: progress and opportunities in polyploid research



The success of cytotype coexistence depends on numerous biological factors and ecological processes, many of which operate at the population level.

Mixed-ploidy species: progress and opportunities in polyploid research

Filip Kolář[1,2], Martin Čertner[1,2], Jan Suda (deceased)[1,2] Peter Schönswetter[3], and Brian C. Husband[4*]

[1] Department of Botany, Faculty of Science, Charles University, Benátská 2, Praha, CZ-128 00, Czech Republic; [2] Institute of Botany, The Czech Academy of Sciences, Zámek 1, Průhonice, CZ-252 43, Czech Republic; [3] Institute of Botany, University of Innsbruck, Sternwartestraße 15, A-6020 Innsbruck, Austria; [4] Department of Integrative Biology, University of Guelph, Guelph, Canada N0B 2K0

**Correspondence: bhusband@uoguelph.ca*

Abstract

Mixed-ploidy species harbour a unique form of genomic and phenotypic variation that influences ecological interactions, facilitates genetic divergence, and offers insight into mechanisms of polyploid evolution. However, there have been few attempts to synthesize this literature. Here we review research on cytotype distribution, diversity and dynamics of intensively studied mixed-ploidy species and consider the implications for understanding mechanisms of polyploidization such as cytotype formation, establishment, coexistence, and post-polyploid divergence. In general, mixed-ploidy species are unevenly represented among families; exhibit high cytotype diversity, often within populations, and frequently comprise rare and odd-numbered ploidies. Odd-ploidies often occur in association with asexuality. We highlight research hypotheses and opportunities that take advantage of the unique properties of ploidy variation.

Keywords

cytotype diversity, establishment, formation, gene flow, genome duplication, reproductive isolation.

Trends

Detailed descriptions of ploidy diversity are published for many mixed-ploidy species. Future advances require robust tests of specific hypotheses pertaining to origin and maintenance of ploidy diversity using experimental and comparative approaches.

The underlying ecological and genetic mechanisms of polyploid evolution have mostly been investigated in a piecemeal fashion. Analyses of multiple factors within one system and comparisons across systems are necessary.

Emerging experimental and molecular evidence suggests that ploidy differences are an imperfect barrier to gene flow. The intensity of gene flow, its causes, asymmetries and variation among polyploid systems require further study.

More emphasis is needed on separating the immediate effects of genome multiplication from selection and evolutionary divergence affecting polyploids once they arise.

Mixed-ploidy species

Polyploidy, the product of whole genome multiplication, represents a unique form of heritable variation with important evolutionary implications due to its high rate of formation [1], potential for genetic/genomic novelty [2], discontinuous phenotypic effects [3] and potential to create reproductive barriers [4]. However, our understanding of the population processes underlying polyploid formation, establishment and persistence, and the contributions of polyploidy to evolutionary divergence are restricted to a few model organisms [5,6].

Mixed-ploidy species, taxa containing at least two cytotypes, offer unique insights into the processes governing polyploid evolution and the role of ploidy variation in adaptation and speciation. Chromosomal surveys estimate that 16% of all plant species contain ploidy variation [7]. Compared to more distant taxonomic comparisons, mixed-ploidy species enable the study of cytotype frequencies and distributions, and their phenotypic, ecological and evolutionary consequences, within a recently shared history and genetic background. In addition, because of their physical proximity, we can directly examine the outcomes of interactions between cytotypes in situ. Arguably, population-level variation in cytotype composition provides a window on the various stages of polyploid evolution from formation to establishment and evolutionary divergence. Despite the significance and research benefits of intraspecific ploidy variation, there have been few attempts to synthesize this literature [8–10].

Here, we review research on the distribution, diversity and dynamics of ploidy mixtures in plants based on a survey of species whose ranges have been extensively sampled for ploidy. We consider the contribution of these patterns for understanding polyploid formation and establishment, ploidy coexistence, and subsequent evolutionary divergence. Through this review, we highlight both recurring patterns and opportunities for future research that take advantage of the unique properties of intraspecific ploidy variation.

Patterns of diversity

We reviewed all published studies of mixed-ploidy species found in the literature that comprised at least 100 individuals (mean N = 1,541 individuals per species) and were screened for ploidy across much of the species' geographic range. In total we reviewed studies for 69 (59 when surveys of just contact zones are excluded) angiosperm species representing 57 genera from 29 families (see online supplemental information, Table S1). These species are not uniformly distributed among families (goodness of fit for five largest families plus rest combined: X^2=167.25, df=5, p<0.0001): 21 species belong to Asteraceae, seven to Poaceae, five to Brassicaceae; eight families are represented by two or three species, and 18 families are each represented by a single species (Table 1).

Table 1 Summary of cytotype diversity in published studies of mixed-ploidy species[a].

Species level:	All species	Mostly sexual	Mostly asexual
# of spp.	59	50	9
# of cytotypes	3.5 (2-8)	3.5 (2-8)	3.0 (2-5)
% rare cytotype individuals	3.0 (0-22)	3.0 (0-22)	2.8 (0-8)
% odd-ploidy cytotype individuals	11.6 (0-70)	4.7 (0-52)	39.1 (5-70)
Population level:	All species	Mostly sexual	Mostly asexual
# of spp.	39	34	5
max # of cytotypes	2.6 (1-5)	2.6 (1-5)	2.4 (1-4)
% pops with multiple cytotypes	16.1 (0-75)	14.6 (0-75)	26.7 (2-64)
% pops with multiple dominant cytotypes	12.0 (0-75)	10.4 (0-75)	24.5 (2-64)
% pops with rare cytotypes[b]	8.9 (0-40)	9.0 (1-40)	6.8 (0-14)
% pops with a dominant and a rare cytotype[b]	6.1 (0-35)	6.6 (0-35)	5.1 (0-10)

[a] Mean values or percentages (range in brackets) at the species level are based on 59 species with ploidy surveys over substantial portions of their geographic range; estimates at the population level are based on 39 species for which at least five (mean = 17) individuals per population were sampled on average. The statistics for species with predominantly sexual and asexual reproduction (apomixis and/or vegetative reproduction prevail in at least one dominant cytotype) are presented separately. Values for ranges are rounded to the nearest whole number.

[b] N = 27, 25 & 2 for all, mostly sexual, and mostly asexual species, respectively, in which at least one rare cytotype was present.

This distribution deviates from expectations based on family species richness ($X^2=0.64 \cdot 10^6$, df=5, $p<0.0001$), and may reflect differences in predisposition for intraspecific ploidy variation among taxonomic groups, not withstanding the potential for study bias. Of the 69 species examined, 40 were identified as autopolyploid (3 allopolypoid; 26 unknown), reflecting the taxonomic practice of retaining morphologically similar cytotypes within the same species [11].

Our survey revealed relatively high cytotype diversity in mixed-ploidy species. On average, species comprised 3.5 cytotypes (range = 2 to 8) (Table 1), more than what is often reported in historical karyological studies for the same species. As an extreme example, *Senecio carniolicus* (now delineated into four species) was originally considered to comprise a single ploidy. The first flow cytometric survey discovered five ploidy cytotypes [12] and a subsequent study with twelve times the sampling intensity revealed eight cytotypes [13].

All but one of the 69 species reviewed comprise multiple dominant cytotypes, defined as having an overall frequency exceeding 20% and sometimes observed in single cytotype populations. The combination of $2x + 4x$ dominant cytotypes was most common (42/69 species), followed by $2x + 4x + 6x$ (6/69, Table 2), likely reflecting the balanced chromosome pairing and higher fertility expected with even numbers of chromosome sets [1]. In addition, most (71%) species contain at least one rare cytotype (Table 2), which may reflect new polyploid origins, between-ploidy hybrids or cytotypes that were once common but have declined in frequency. These cytotypes, which comprise ~3% of all individuals per species (Table 1), contribute markedly to ploidy richness (up to five rare cytotypes per species; Table 2) and can disproportionately influence cytotype dynamics through reproductive interactions and gene flow [14] (Fig. 1).

Dominant cytotypes are rarely entirely spatially isolated (allopatry, 4% of species). More often, they form contact zones spanning limited integration of their ranges (large-scale parapatry) to more extensive integration with either spatial intermingling of mostly single cytotype populations (mosaic parapatry) or frequent coexistence in mixed-ploidy populations (sympatry; see details in Fig. 2B). Mixtures of dominant cytotypes within populations occur in most range-wide surveys (86%) and constitute a mean of 12% of all populations per species (Table 1). Rare cytotypes add to that diversity within 8.9 % of populations; 6.1% of populations per species containing both a dominant and a rare cytotype (Table 1). It is less clear whether within-population cytotype diversity is generated locally (i.e. primary contact zone) or arises through migration after allopatric divergence (secondary contact) (Fig. 1A) [9]. Most phylogeographic evidence supports secondary contact, e.g., [15–18] (Table 3); primary contact between diploids and their autopolyploid derivatives is supported by close multilocus allozyme or microsatellite profiles in three examples [19–21] and both pathways appear to operate in different populations of another species as suggested by nearly identical diploid vs. tetraploid AFLP profiles

Table 2 Ploidy combinations reported among 69 mixed-ploidy plant species[a].

Dominant cytotypes	# species	% mostly asexual species	% species with odd cytotype(s)	% species with intermediate cytotype(s)	% species with rare cytotypes	rare cytotypes present (# of cases)				
						3x	4x	5x	6x	other cytotypes (# of cases if > 1)
$2x+3x$	4	100	100	0	25	-	1	0	0	
$2x+4x$	42	2	62	62	67	26	-	5	12	
$2x+6x$	2	0	50	0	50	1	0	1	-	$7x$,$8x$, $9x$
$2x+3x+4x$	2	50	100	100	100	-	-	2	0	
$2x+4x+6x$	6	0	83	83	83	4	-	5	-	$7x$ (2), $8x$ (2), $9x$
$4x+5x+6x$	2	100	100	100	100	1	-	-	-	$7x$
$4x+6x$	2	0	50	50	50	0	-	1	-	
$4x+8x$	3	0	0	100	100	0	-	0	3	
others[b]	6	17	83	50	100	3	0	1	1	$7x$ (2), $8x$ (2), $9x$, $10x$ (2), $11x$, $12x$, $14x$

[a] All species included had >100 plants measured for ploidy. Assessments of rare, odd and intermediate ploidy cytotypes are based on adult plants. See Table S1 for details on individual systems.

[b] Ploidy combinations (1 case each): $2x+4x+5x$ + rare $3x$; $2x+4x+6x+12x$ +rare $3x$; $4x+6x+8x$ + rare $3x$, $5x$, $7x$; $4x+12x$ + rare $6x$, $10x$, $14x$; $6x$ + rare $8x$, $9x$, $10x$, $11x$, $12x$; $6x+9x$ + rare $7x$, $8x$

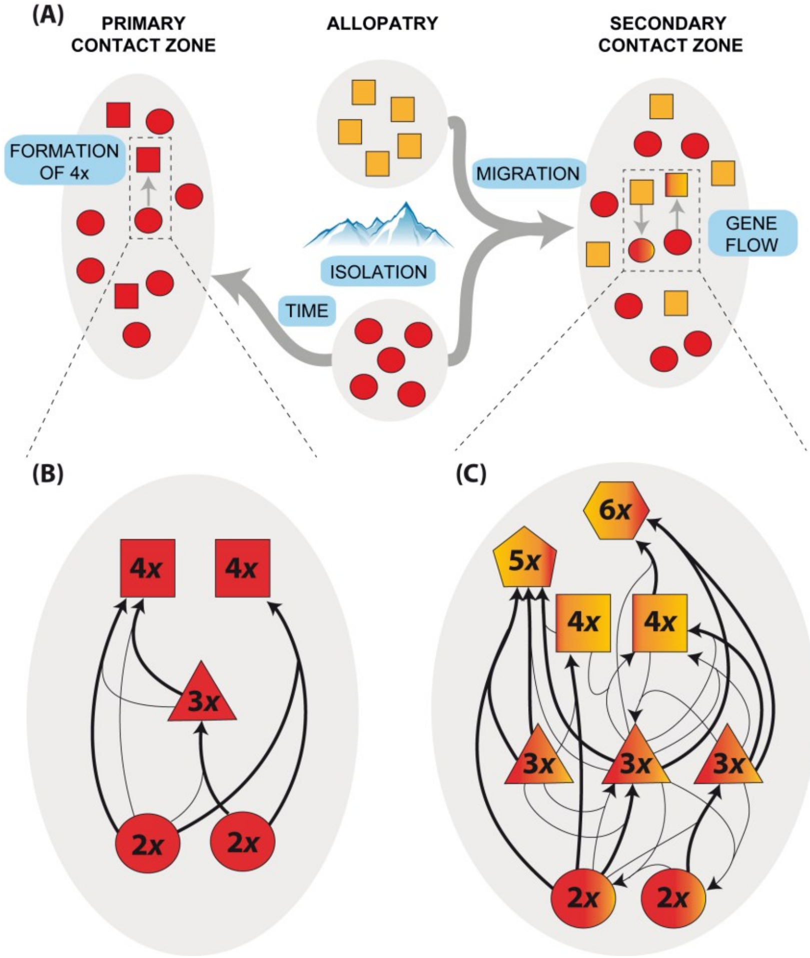


Figure 1 Spatiotemporal evolution of cytotype diversity in a predominantly diploid-tetraploid species. (A) Two dominant cytotypes ($2x$ and $4x$) come into contact either through novel *in situ* formation and establishment of tetraploids from diploids (primary contact) or co-migration from spatially segregated and divergent populations (secondary contact). With primary contact, both cytotypes have similar (red) genetic backgrounds, whereas in secondary contact they are genetically differentiated (red and orange) by means of genetic drift and/or local adaptation. (B) In situ formation of tetraploid cytotypes via unreduced gametes through either unilateral (triploid bridge: $2x \times 2x = 3x$, then $3x \times 2x = 4x$) or bilateral ($2x \times 2x = 4x$) pathways. (C) Cytotype interactions within secondary contact zone resulting in rare cytotypes and across-ploidy gene flow (colour gradients). Reduced (thin lines; x and $2x$ in triploid) and unreduced gametes (thick lines; $3x$ in triploid) are depicted by lines. Note the important role of intermediate ploidy (triploids, 3x) as a mediator of gene flow. For simplicity we excluded rare pathways involving aneuploidy, reduction of ploidy state through haploid parthenogenesis, merger of two unreduced gametes of even-ploidy cytotypes, reproductive interactions between the rare cytotypes and formation of cytotypes higher than hexaploid.

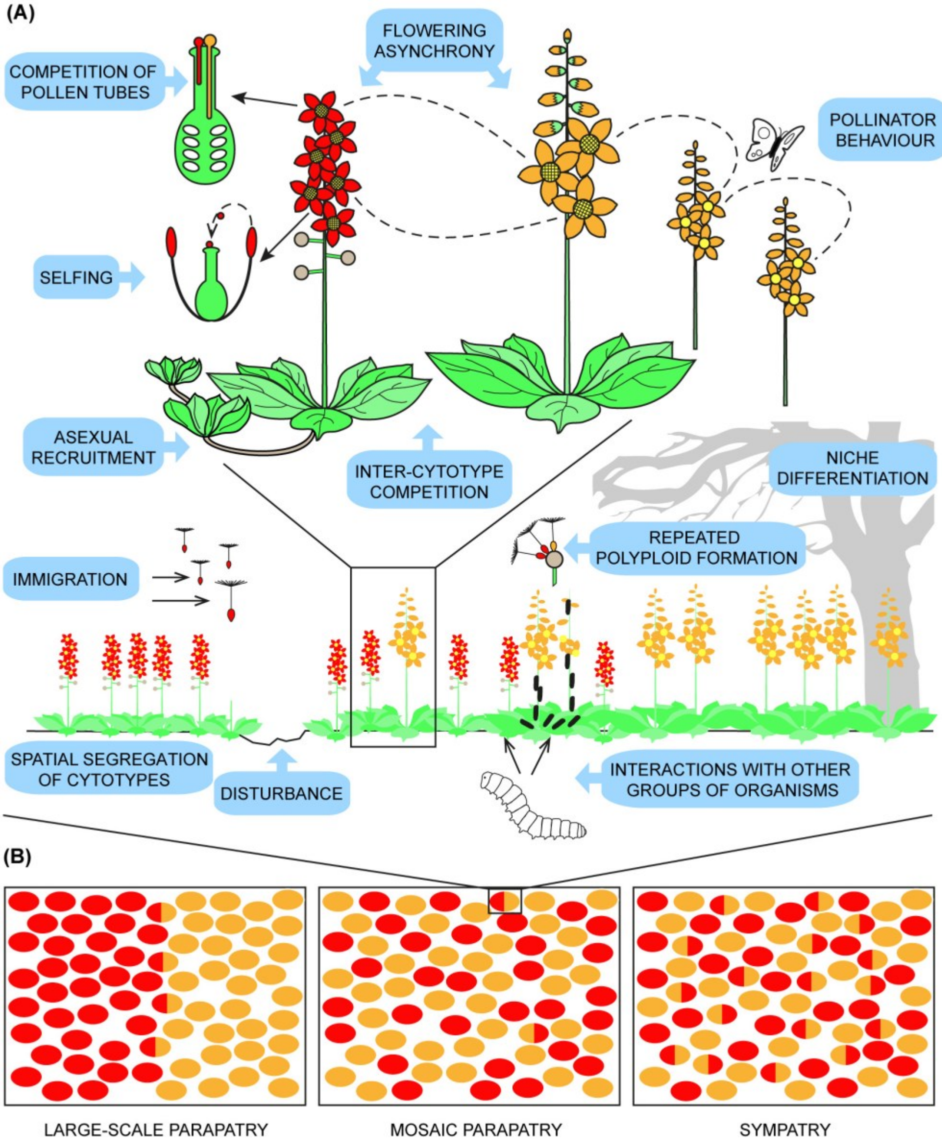


Figure 2 Spatial patterns and mechanisms affecting cytotype coexistence in mixed ploidy species. (A) Mechanisms facilitating cytotype coexistence within a mixed-ploidy population of diploid (red-flowered) and tetraploid (orange-flowered) individuals. (B) Spatial arrangement of cytotypes within and among populations at the landscape level, ranging from uniform-ploidy areas with a narrow contact zone (left panel, 39% of widely screened species), a diffuse contact zone with predominantly uniform-ploidy populations and rare ploidy mixtures (middle panel, 30% of species) to a diffuse, mosaic-like contact zone with both single cytotype and mixed-ploidy populations (right panel, 27% of species)(NB: allopatry was detected in remaining 4% of cases).

compared to divergent profiles in other parts of the species' range [22]. The history of mixture has important implications for interpreting the mechanisms and timing of polyploid divergence.

Many species contain odd ploidy cytotypes (3*x*, 5*x*, 7*x*, 9*x* etc.), which can play an important role in polyploid evolution as mediators of gene flow and recurrent polyploid origins (Fig. 1C). They occur in 65% (45/69) of species, often as intermediates between dominant ploidies, and comprise, on average, 11.6% of samples (this value is raised by several asexual species with odd-ploidy dominant cytotypes, Table 1). The hybrid origin of these cytotypes has occasionally been confirmed through seed ploidy analyses [23,24], but detailed genetic studies of their role in gene flow and higher polyploid origins are limited [15,17,18,25].

Polyploid origin and formation

The diversity of cytotypes within mixed-ploidy species (Table 1, 2) implies that the rate of new polyploid origins exceeds the rate of extinction. This circumstance is ideal for studying the pathways and rates of polyploid formation, which dictate the likelihood of establishment and its genetic consequences. Some of the strongest evidence for polyploid origins comes from inferences using molecular markers [22,26,27], which show that polyploid lineages have arisen and persisted recurrently in many taxa [28]. However, we know little about the actual (vs realized) rates of neopolyploid formation and the specific mechanisms (unreduced gametes, somatic doubling, hybridization) or pathways (unilateral, bilateral) involved (Table 3).

The incidence of rare cytotypes among seeds or adults in otherwise uniform populations suggests that *in situ* formation of polyploids may occur frequently. For example, rare triploids or occasional tetraploids are observed within diploid populations (e.g., [23,26,29]), in offspring from mothers of different ploidy [24], or in experimental crosses [30–32]. Combined with data on ploidy fitness and mating patterns, this evidence has led to the suggestion that polyploids arise spontaneously at surprisingly high rates [1]. Screening for new polyploids in natural populations, or identifying unique polyploid lineages using markers, remains a laudable goal, but is limited by the need for high sampling intensity, sufficient differentiation among separate polyploid origins at genetic markers (genes or genome size)[33] and statistical tools for distinguishing newly produced (first 1-2 generations) from established polyploids (generated through matings among polyploids). Advances in these areas may also enable researchers to quantitatively distinguish between the various pathways (bilateral vs unilateral) and mechanisms (unreduced gametes vs somatic doubling) of polyploid formation (Fig. 1B).

Mixed-ploidy species provide suitable systems for studying rates and determinants of unreduced gamete production, widely believed to be the most common cause of polyploid formation. While the presence of unreduced gametes

Table 3 Summary of major research emphases (and gaps, marked by a dash) for the 25 most comprehensively studied mixed-ploidy plant species.

Species[a]	Family	Cytotypes (x)	Geographic distribution	Formation	Spatial isolation	Assort. mating	Compe-tition	Gene flow	Evol. history	Selection after WGD
Achillea borealis	Asteraceae	4,6	s	r+p	y / -	y	-	-	y	y
Allium oleraceum	Amaryllidaceae	4,5,6 (3)	s (+?p)	p	n / y	(y)[b]	n	y	y	-
Andropogon gerardii	Poaceae	6,9 (7 or 8)	-	p	y / y	-	(y)	(y)	-	-
Arnica cordifolia	Asteraceae	(2),3,4 (5)	-	-	y / y	y[b]	-	y (low)	-	-
Arrhenatherum elatius	Poaceae	2,4 (3)	s	-	y / y	y	y	n	-	y
Aster amellus	Asteraceae	2,6 (3,5,7,8,9)	s	-	y / n	n	n	n	y	-
Butomus umbellatus	Butomaceae	2,3	s	p	y / -	(y)[b]	y	y (low)	y	-
Centaurea phrygia	Asteraceae	2,4 (3,5,6)	s	r	y / -	-	-	y (low)	-	-
Centaurea stoebe	Asteraceae	2,4 (3)	s	p	y / y	n	(y)	n	y	-
Chamerion angustifolium	Onagraceae	2,4 (3,5)	s	r+p	y / y	y	n	y (low)	-	y
Dactylis glomerata	Poaceae	2,4	p	p	n / y	(y)	y	-	-	-
Gymnadenia conopsea	Orchidaceae	2,4 (3,5,6)	-	-	n / y	y/n[c]	-	-	-	-
Heuchera grossulariifolia	Saxifragaceae	2,4 (3)	s	p	y / -	y	-	y (low)	y	y
Knautia arvensis	Caprifoliaceae	2,4 (3)	s+p	p	y / y	(n)	(n)	n	y	-
Larrea tridentata	Zygophyllaceae	2,4,6 (3,5)	s	p	y / y	y	-	y	y	-
Mercurialis annua	Euphorbiaceae	2,6 (4,8)	s	-	y / -	-	y	-	-	-
Oxalis pes-caprae	Oxalidaceae	2,4,5 (3)	s	p	n / -	(y)[b]	-	-	y	-
Pilosella echioides	Asteraceae	2,4 (3,5,6)	s (+?p)	r+p	y / y	y/(n)[d]	-	y	-	-
Plantago media	Plantaginaceae	2,4 (3)	s	-	y / -	(y)	-	n	y	y
Ranunculus adoneus	Ranunculaceae	2,4 (3,6)	s	p	y / y	n	(n)	-	-	-
Ranunculus kuepferi	Ranunculaceae	2,4 (3,5,6)	s	p	y / -	(y)[b]	-	n	y	-

Table 3 (continued)

Species [a]	Family	Cytotypes (x)	Geographic distribution	Formation	Spatial isolation	Assort. mating	Compe-tition	Gene flow	Evol. history	Selection after WGD
Senecio carniolicus	Asteraceae	2,4,6 (3,5,7,8,9)	s	p	y / y	n	(y)	y (low)	y	-
Solidago altissima	Asteraceae	2,4,6	(?s)	-	y / n	-	n	-	y	-
Tripleurospermum inodorum	Asteraceae	2,4 (3,5,6)	s	r+p	y / y	-	-	-	-	-
Vicia cracca	Fabaceae	2,4 (3)	s	p	y / y	-	(y)	n	-	-
# of species studied (only tested cases)	-	25	21	18	25 / 17	12	15	16	14	5

[a] This list is a selected subset of the original 69 species that examine multiple aspects and hypotheses related to ploidy coexistence. See online supplemental information, Table S1, for details on the particular study categories and species as well as the original references. Cytotype refers to all dominant and rare (brackets) ploidy levels observed for a given species. Current research is described in terms of evidence available with respect to geographical distribution of dominant cytotypes (s = secondary contact, p = primary contact); formation (r = rate, p = pathways was addressed); spatial isolation at regional / intrapopulation scales (y=evidence for, n = evidence against); assortative mating (excluding spatial isolation; y=evidence for, n = evidence against); differentiation in competitive abilities of the cytotypes (y = evidence for, n = evidence against); gene flow indicated by molecular markers / detailed intrapopulation cytotype screenings (y = evidence for, n = evidence against); evolutionary history of the polyploid is known (y = yes, known); selection after WGD (y = evidence for). Dash (-) indicates no research is available for a given topic, (y) / (n) means that presence / absence of the role of particular aspect was indicated but not specifically tested.

[b] at least some cytotypes asexual

[c] phenology shift among one group of 2*x* and 4*x*, but no pollinator preferences among other group of 2*x* and 4*x* with flowering synchrony

[d] polyploids are sexual in the intensely studied sympatric populations but agamospermous in another part of the range

has been noted in many species, their frequency in natural populations has been estimated in few species, mixed ploidy or otherwise (e.g., [32,34,35]). These studies reveal low but variable rates of unreduced gamete production and a weak correlation between unreduced ovules and pollen among individuals [34,36,37]. Both parameters are critical for understanding the potential for, and genetic consequences of, polyploid formation. Moreover, it will be important to relate unreduced gamete production to realized frequencies of polyploid formation by assessing ploidy in offspring from known ploidy cross combinations [30–32,38,39] and measuring the mating success of unreduced gametes in competition with reduced gametes [40,41]. A stronger focus on the mechanisms and rates of polyploid formation in natural conditions will be critical for explaining variation in polyploid diversity among plant taxa under specific ecological conditions and geological time periods [42].

Polyploid establishment

Assuming random mating, new polyploids should be rapidly eliminated from populations of their progenitors due to a frequency-dependent mating disadvantage, referred to as minority cytotype exclusion (MCE)[43]. This process, resulting from elevated intercytotype mating leading to low offspring fitness, is a theoretical obstacle to establishment of new polyploids [44]. Less clear is what the magnitude of MCE is in natural populations, although the abundance of mixed-ploidy species and populations is itself circumstantial evidence that the barriers to new polyploids can be overcome. Studies such as [45] observed no reduction in fitness of rare cytotypes in natural populations of a facultatively asexual species, whereas experimental investigations of sexual species [46,47] indicate that minority effects can reduce the fitness of polyploids and may account for dominance of certain cytotypes in some locations. Additional experiments involving ploidy composition, frequency and spatial configuration, and plants with diverse life histories, will help to fully understand in which species MCE will be enhanced or diminished.

New cytotypes can establish in the face of MCE through several mechanisms: I) repeated formation of polyploids [44] and demographic stochasticity [43], both of which can elevate the frequency of polyploids; II) assortative (within-ploidy) mating and asexual recruitment, which minimize the frequency of inter-cytotype mating [43,48]; and, III) increased competitive ability and fecundity of polyploids, which can compensate for the frequency-dependent disadvantage [49,50]. The incidence and relative strength of processes in category II, especially assortative mating, have been well explored using mixed-ploidy species (see below) whereas mechanisms associated with categories I and III have received less attention.

Repeated polyploid formation can contribute to establishment when the rates of origin exceed the loss due to minority exclusion [44]. Nevertheless, quantitative estimates of de novo polyploid formation are limited, largely because of the requirement for high throughput screening of offspring ploidy from mothers of known ploidy [35] or high-resolution genotyping to distinguish individual origins (see section on *Polyploid origins and formation*). Where estimates exist, rates of polyploid formation can account for the presence of cytotype mixtures but are not sufficient to account for persistence of polyploids, which requires high rates (6% - 17%) depending on relative viability and fecundity of polyploids [44]. Similarly, demographic stochastic forces may overcome MCE by causing random increases or fixation of rare polyploids [43]. To our knowledge, there have been no attempts to relate cytotype diversity to determinants of genetic drift such as population size and founder events in natural populations.

Assortative mating in mixed-ploidy species can be achieved through multiple mechanisms including spatial segregation of cytotypes, asynchronous flowering, and assortative pollen transfer (Fig. 2A). Spatial segregation of intraspecific cytotypes is widely reported on regional [13,23,24] and local [51–54] spatial scales. The underlying causes of such separation include niche differentiation [55–57], colonization history [15,22,58], limited dispersal, and clonal expansion [54,59,60]. Niche differentiation is considered prevalent but is difficult to demonstrate without experimental comparisons of plant fitness in contrasting environments [55,61–64]. When available, such common garden studies corroborate the presence of cytotype differentiation but the direction of divergence is rarely consistent. Associations between ploidy distributions and climate are also suggestive of niche differentiation, although there appears to be no consistent pattern of niche shift in mixed-ploidy species [18,62,65,66] and niche is largely defined by climatic rather than other local environmental variables (but see [65]). Future work will need to test for habitat segregation at multiple spatial scales [54,67] and using multiple environmental parameters [68] coupled with reciprocal transplant and common garden comparisons.

Numerous mechanisms of assortative pollination and fertilization have also been examined in mixed-ploidy species (Fig 2A) ranging from flowering asynchrony [56,63,69], pollinator differentiation and foraging fidelity [70–72] to selfing [73,74] and homoploid pollen precedence [40]. While individual mechanisms such as flowering time are measured in extant cytotypes with regularity, estimates of multiple mechanisms in a single system are not common (but see [15,48,69,75]; Table 3). The latter studies suggest that multiple factors can contribute to assortative mating but the importance of particular barriers is likely to be species-specific (reviewed in [76]). Despite being one of the most intensively studied topics, research opportunities remain including comparisons across species, measures of the contribution of assortative pollination to genetic isolation, and examination of direct effects of genome duplication using synthetic polyploids [75].

Asexual reproduction (apomixis, vegetative reproduction) can facilitate establishment by reducing between-cytotype mating and allowing rare cytotypes to persist without sexual recruitment [77,78]. Strong evidence for the importance of asexuality comes from our survey of mixed-ploidy species (Table 1). The occurrence of odd-ploidy cytotypes, which are generally sexually compromised, is strongly and positively associated with asexuality, as is within-population cytotype diversity (Table 1). Further, species in which odd-ploidy cytotypes are dominant exhibit strong if not exclusively asexual modes of reproduction (e.g., [45,79,80]. The questions that remain are I) how does (facultative) asexuality interact with patterns of sexual reproduction to influence the dynamics of mixed-ploidy populations [81], II) can asexuality favour long-term (stable) coexistence of cytotypes or does it simply delay the sorting of cytotypes through frequency-dependent sexual processes or competitive interactions, and III) does selection strengthen relative allocation of reproductive effort to asexuality after polyploidization? Additional research on the relations between asexual recruitment and ploidy diversity, fitness of clones of different size and spatial configuration, and differences in capacity for asexual reproduction among cytotypes [58,60,82] will be key for addressing these questions.

Minority cytotype exclusion can be counteracted to some extent by increased viability, competitive ability and/or fecundity of new polyploids relative to their progenitors [44]. Polyploid competitive advantage must be particularly strong to balance the strength of minority disadvantage unless it is operating in conjunction with other factors. Experimental studies of relative fitness and competitive ability of polyploids are uncommon [36,55,83,84]. In most cases, polyploids do not have greater competitive ability although comparisons involving neopolyploids in a diverse set of environments and longterm observations of cytotype frequencies are still needed. Researchers have tested for ploidy-specific differences in effects of herbivores, parasites, mutualists [76] (Fig 2) but the relation to relative viability and fecundity is less clear. Overall, results to date suggest that viability and fecundity differences alone are unlikely to overcome the impact of minority cytotype exclusion.

Stable coexistence

The prevalence of cytotype co-occurrence (Table 1) raises the question of whether mixed-ploidy populations are stable over the long term or in a transient state of coexistence. As with other forms of taxonomic diversity, long term coexistence of cytotypes may be favoured by the presence of ecological trade-offs and niche differentiation (see *Polyploid establishment* above; [57]), recurrent migration among populations, and disturbance, which can promote spatially or temporally heterogeneous environments [15,85]. Of these mechanisms, the contribution of niche differentiation to coexistence has received most attention in the context of cytotype diversity (see *Polyploid establishment*; Fig. 2A). The roles of recurring migration among populations and disturbance in maintaining cytotype diversity are less clear [86]. In one case, Mráz et al. [15] present evidence that human-mediated disturbance generates environmental variation, which enables tetraploid cytotypes of *Centaurea stoebe* to co-exist with diploids in spatially segregated microhabitats. Experimental approaches will be particularly useful for determining whether disturbance itself favours polyploids or whether it maintains cytotype diversity by creating environmental heterogeneity.

In addition, two attributes specific to polyploid systems may promote long-term coexistence of cytotypes. First, recurring and frequent polyploid formation (see *Polyploid origin and formation*) allows multiple cytotypes to persist in dynamic balance with the forces of minority exclusion [44]. Equally important, production of multiple gamete ploidies per individual [38,71,87] and mixed mating within and between cytotypes [24] can generate multiple cytotypes each reproductive cycle. For example, diploid individuals can produce viable x and $2x$ gametes, and triploids may produce x, $2x$ and $3x$ gametes, which can unite to maintain cytotype diversity in the face of cytotype exclusion [14] (Fig. 1C). Analyses of the mating patterns and outcome of between-ploidy crosses of mixed-ploidy species beyond a few well-studied systems are needed to reveal the importance of this process [39,88].

Demonstrating stability of cytotype diversity requires data on temporal changes in cytotype composition but examples are rare. Studies on *Mercurialis annua* [61] after four decades, and of *Tripleurospermum inodorum* [33] over a few years, document significant shifts in cytotype composition, occasionally leading to displacement or extirpation of one cytotype. In contrast, the distribution and size of hexaploid and enneaploid tussocks of *Andropogon gerardii* remained stable over four years [89] and no signs of cytotype fixation were observed in sequential surveys in other perennial, mixed-ploidy species [59,62,90]. Analysis of seed morphology in sediment and peat, or leaf anatomy in fossil material may provide a long term perspective on dynamics of cytotype diversity in some systems.

Polyploid divergence after genome duplication

Mixed-ploidy species provide opportunities to distinguish the immediate (direct) effects of genome duplication on phenotypic, ecological, and genomic divergence from indirect effects due to divergent selection after polyploid formation. Differences between extant diploids and polyploids are often interpreted as the immediate effects of genome duplication. However, for all but the youngest polyploids, selection after the duplication event is likely to be a significant contributor to divergence. Ignoring this process can lead to misinterpretations about the role of polyploidy in phenotypic divergence and the mechanisms facilitating polyploid establishment.

Insights into the direct effects of polyploidy are emerging from phenotypic and genomic analyses of recently formed natural polyploids. A number of examples exist of polyploids arising in the last 150 years, such as *Spartina anglica* [91], *Tragopogon mirus* and *T. miscellus* [92], *Mimulus peregrinus* [93] and *Senecio cambrensis* and *S. eboracensis* [94]. All of these cases are examples of allopolyploids, which confound the effects of genome duplication with interspecific hybridization. Recently formed spontaneous autopolyploids are less reported but are needed to isolate the initial effects of whole genome duplication. Species forming primary contact zones may be fruitful targets for this approach, since progenitor and polyploid derivatives share a recent phylogenetic history [54].

An alternate strategy for evaluating the immediate effects of polyploidy is to experimentally generate new polyploids, through union of unreduced gametes or transformation by exposure to cytokinesis disruptors (e.g. colchicine). If such neopolyploids resemble naturally occurring polyploids, one can infer that immediate effects of genome duplication are substantial. This tool has been used extensively in genomic research [95,96] but less often in ecological contexts (29,62,74,96]), likely because of the challenges generating multiple neopolyploid lines to reflect natural diversity and ethical concerns with using them in field settings. When achieved, such studies suggest that no more than half of phenotypic divergence results from the direct effects of whole genome duplication [63,75]. Less clear is which traits are likely to be most affected and in what manner.

Equally important is the need to understand the patterns of selection affecting cytotypes after genome duplication. A newly formed polyploid likely faces selection to stabilize plant viability and fertility affected by increased chromosome number and gene dosage. Measures of the magnitude and directions of selection are rare although recent studies have documented genomic signals of selection on genes linked with meiosis in an autotetraploid [98] and on recombination of subgenomes in allopolyploids [99,100].

Selection mediated by ploidy mixture may also maximize within-cytotype mating and minimize competitive and reproductive interactions with diploid progenitor (i.e., character displacement, reinforcement) in mixed-ploidy populations. Observations of phenotypes in allopatric versus sympatric cytotypes suggest that ploidy interactions can enhance divergent selection [76]). For example, Petit et al. [101] observed greater selfing rates in parapatric populations of diploid and tetraploid *Arrhenatherum elatius* compared to allopatric sites, consistent with reinforcement selection. Sonnleitner et al. [57] found stronger niche differentiation among three cytotypes of *Senecio carniolicus* in sympatry than in allopatry. Similarly, Nuismer and Cunningham [102] found evidence of reinforcement in mixed ploidy *Heuchera grossulariifolia* using measures of phenotypic selection. Further research is needed that accounts for confounding environmental differences between sympatric and allopatric populations, includes neopolyploids, and integrates ecological, cytological and genomic approaches to evaluating patterns of selection.

Concluding Remarks and Future opportunities

Detailed descriptions of cytotype diversity and distribution are now available for many mixed-ploidy species. These studies reveal a high frequency of cytotype diversity and coexistence, often within populations, and a strong association between diversity and mode of reproduction. Such patterns raise many hypotheses as to how this diversity arises and is maintained in natural populations, and how genome duplication contributes, relative to genic diversity, to evolutionary divergence in plants.

To advance our general understanding of evolution in polyploid systems, future studies of mixed-ploidy species can contribute in several ways. First, detailed descriptions of cytotype diversity and distribution of allopolyploids and their progenitors must keep pace with studies of putative autopolyploids. Ironically, allopolyploids have been the primary focus of genomic research yet their population biology is less well documented. Second, research should increasingly take advantage of mixed cytotype populations to conduct robust tests of hypotheses through experimental and comparative approaches. Third, given the patterns observed here, more work, from a theoretical, experimental and comparative perspective should explore the underlying causes for the associations between asexuality and polyploidy. Finally, we advocate the value of integrating the extensive literature to develop a more holistic perspective of polyploid evolution. Suda and Herben [103] and Oswald and Nuismer [97] are good examples of models that are parameterized by using the data available to infer processes less well studied or to evaluate the relative importance of different mechanisms.

Outstanding Questions

What predisposes some taxa to exhibit intraspecific ploidy variation but not others? Future comparisons of key phenotypic and genetic features related to polyploid formation and establishment in sister taxa that differ in ploidy variation are needed to address this long-standing problem.

What are the rates of unreduced gamete production and pathways of polyploid formation in natural populations? Studies using genetic markers and high-throughput ploidy screening are required to improve our understanding of the ecological and evolutionary mechanisms favouring polyploid formation.

*What is the contribution of diploid-polyploid hybrids and odd-ploidy cytotypes to the generation and maintenance of mixed-ploidy populations***?** Do rare cytotypes have a disproportionate influence, and can diversity escalate in a positive feedback process?

How stable are mixed-ploidy populations? Do they arise and disappear transiently or are they stable over long periods of time? Studies of temporal variation in cytotype composition of ploidy mixtures are feasible with today's technologies.

What are the immediate effects of whole genome multiplication on evolutionary divergence? Further use of neopolyploids (natural or synthetic) is needed to understand how abrupt speciation is and how these saltation events in turn affect selection on ploidy cytotypes.

How does asexual reproduction influence polyploid evolution, before or after the duplication event? Phylogenetic comparative analyses among multiple species, experimental studies and theoretical models are needed to explain cytotype diversity in largely asexual species and their role in polyploid formation and establishment.

What forms and agents of selection influence newly formed polyploids? Phenotypic and genomic studies will be important for evaluating the roles of reinforcement, ecological character displacement and frequency-dependent selection in mixed ploidy populations and identifying genomic regions exhibiting signals of past selection.

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Glossary

allopolyploid: polyploid formed from multiplication of genomes (> 2 copies) derived from different taxonomic species

autopolyploid: polyploid formed from the multiplication of genomes (> 2 copies) within a single taxonomic species

bilateral pathway: formation of a polyploid from a diploid through the union of two unreduced gametes

cytotype: chromosomal variant within a species. In the context of this paper, refers to multiples of the monoploid chromosome number (i.e. ploidy cytotype; e.g., diploid, 2*x*; triploid, 3*x*; tetraploid, 4*x*; etc.)

minority cytotype exclusion: the elimination of rare cytotypes in a population due to reduced fitness and production of hybrid cytotypes as a result of mating with the majority cytotype

mixed-ploidy: having more than one ploidy cytotype per species or population within species

polyploid: individual with more than two complete chromosome sets (or genomes) per nucleus

population: in the context of this paper, a geographically discrete sampling location

primary contact zone: geographic area within which a polyploid arises and coexists with its direct progenitor(s) (diploid, lower level polyploid) *secondary contact zone*: geographic area within which a polyploid exists in close proximity to its progenitor(s) (diploid or lower level polyploid) but where the polyploid arose elsewhere but arrived by range expansion and is thus genetically divergent from its co-occurring progenitor(s)

dominant cytotype: a ploidy cyotype whose frequency is > 20% and which can occur in single-cytotype populations

rare cytotype: globally rare cytotype which never forms uniform populations; typically an infrequent product of between-ploidy hybridization or reproduction involving unreduced gametes

intermediate cytotype: cytotype numerically midway between dominant ploidy levels in a species; often resulting from between-ploidy hybridization

unilateral polyploid pathway: formation of polyploids through union of a reduced and unreduced gamete, to produce an intermediary polyploid, which then backcrosses to a diploid

unreduced gamete: egg or sperm nuclei with the somatic ($2n$) chromosome number

unbalanced gamete: egg or sperm nuclei with more or fewer chromosomes than a complete chromosome set

triploid block: strong inviability of triploid cytotype relative to its diploid and tetraploid parents

triploid bridge: formation of tetraploids from diploids via a fertile triploid cytotype, which backcrosses to diploids (see unilateral polyploid pathway)

References

1 Ramsey, J. and Schemske, D.W. (1998) Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annu. Rev. Ecol. Syst.* 29, 467–501

2 Adams, K.L. and Wendel, J.F. (2005) Polyploidy and genome evolution in plants. *Curr. Opin. Plant Biol.* 8, 135–141

3 Levin, D.A. (2002) *The role of chromosomal change in plant evolution*, Oxford University Press.

4 Coyne, J.A. and Orr, H.A. (2004) *Speciation*, Sinauer.

5 Soltis, D.E. *et al.* (2016) Polyploidy: Pitfalls and paths to a paradigm. *Am. J. Bot.* 103, 1146–1166

6 Ramsey, J. and Ramsey, T.S. (2014) Ecological studies of polyploidy in the 100 years following its discovery. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 369, 20130352

7 Rice, A. *et al.* (2015) The Chromosome Counts Database (CCDB) – a community resource of plant chromosome numbers. *New Phytol.* 206, 19–26

8 Thompson, J.D. and Lumaret, R. (1992) The evolutionary dynamics of polyploid plants: origins, establishment and persistence. *Trends Ecol. Evol.* 7, 302–307

9 Petit, C. *et al.* (1999) Evolutionary consequences of diploid–polyploid hybrid zones in wild species. *Trends Ecol. Evol.* 14, 306–311

10 Soltis, D.E. *et al.* (2010) What we still don't know about polyploidy. *Taxon* 59, 1387–1403

11 Soltis, D.E. *et al.* (2007) Autopolyploidy in angiosperms: have we grossly underestimated the number of species? *Taxon* 56, 13–30

12 Suda, J. *et al.* (2007) Complex distribution patterns of di-, tetra-, and hexaploid cytotypes in the European high mountain plant *Senecio carniolicus* (Asteraceae). *Am. J. Bot.* 94, 1391–1401

13 Sonnleitner, M. *et al.* (2010) Distribution and habitat segregation on different spatial scales among diploid, tetraploid and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps. *Ann. Bot.* 106, 967–977

14 Husband, B.C. (2004) The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. *Biol. J. Linn. Soc.* 82, 537–546

15 Mráz, P. *et al.* (2012) Anthropogenic disturbance as a driver of microspatial and microhabitat segregation of cytotypes of *Centaurea stoebe* and cytotype interactions in secondary contact zones. *Ann. Bot.* 110, 615–627

16 Eidesen, P.B. *et al.* (2013) Tetraploids do not form cushions: association of ploidy level, growth form and ecology in the High Arctic *Saxifraga oppositifolia* L. s. lat. (Saxifragaceae) in Svalbard. *Polar Res.* 32, 20071

17 Hülber, K. *et al.* (2015) Ecological differentiation, lack of hybrids involving diploids, and asymmetric gene flow between polyploids in narrow contact zones of *Senecio carniolicus* (syn. *Jacobaea carniolica*, Asteraceae). *Ecol. Evol.* 5, 1224–1234

18 Zozomová-Lihová, J. *et al.* (2015) Cytotype distribution patterns, ecological differentiation, and genetic structure in a diploid–tetraploid contact zone of *Cardamine amara*. *Am. J. Bot.* 102, 1380–1395

19 Lumaret, R. and Barrientos, E. (1990) Phylogenetic relationships and gene flow between sympatric diploid and tetraploid plants of *Dactylis glomerata* (Gramineae). *Plant Syst. Evol.* 169, 81–96

20 Šingliarová, B. *et al.* (2011) Biosystematic study of the diploid-polyploid *Pilosella alpicola* group with variation in breeding system: Patterns and processes. *Taxon* 60, 450–470

21 Simón-Porcar, V.I. *et al.* Recent autopolyploidization in a naturalized population of *Mimulus guttatus* (Phrymaceae). *Bot. J. Linn. Soc.* DOI: 10.1093/botlinnean/box052

22 Kolář, F. *et al.* (2012) Bringing together evolution on serpentine and polyploidy: spatiotemporal history of the diploid-tetraploid complex of *Knautia arvensis* (Dipsacaceae). *PLoS ONE* 7, e39988

23 Kolář, F. *et al.* (2009) Towards resolving the *Knautia arvensis* agg. (Dipsacaceae) puzzle: primary and secondary contact zones and ploidy segregation at landscape and microgeographic scales. *Ann. Bot.* 103, 963–974

24 Sabara, H.A. *et al.* (2013) Cytotype coexistence leads to triploid hybrid production in a diploid–tetraploid contact zone of *Chamerion angustifolium* (Onagraceae). *Am. J. Bot.* 100, 962–970

25 Winkler, M. *et al.* (2017) A novel method to infer the origin of polyploids from Amplified Fragment Length Polymorphism data reveals that the alpine polyploid complex of *Senecio carniolicus* (Asteraceae) evolved mainly via autopolyploidy. *Mol. Ecol. Resour.* DOI: 10.1111/1755-0998.12641

26 Mandák, B. *et al.* (2016) Flow cytometry, microsatellites and niche models reveal the origins and geographical structure of *Alnus glutinosa* populations in Europe. *Ann. Bot.* 117, 107–120

27 Španiel, S. *et al.* (2011) Genetic and morphological variation in the diploid–polyploid *Alyssum montanum* in Central Europe: taxonomic and evolutionary considerations. *Plant Syst. Evol.* 294, 1–25

28 Soltis, D.E. and Soltis, P.S. (1999) Polyploidy: recurrent formation and genome evolution. *Trends Ecol. Evol.* 14, 348–352

29 Arnold, B. *et al.* (2015) Single geographic origin of a widespread autotetraploid *Arabidopsis arenosa* lineage followed by interploidy admixture. *Mol. Biol. Evol.* 32, 1382–1395

30 Bretagnolle, F. and Lumaret, R. (1995) Bilateral polyploidization in *Dactylis glomerata* L. subsp. lusitanica: occurrence, morphological and genetic characteristics of first polyploids. *Euphytica* 84, 197–207

31 Burton, T.L. and Husband, B.C. (2001) Fecundity and offspring ploidy in matings among diploid, triploid and tetraploid *Chamerion angustifolium* (Onagraceae): consequences for tetraploid establishment. *Heredity* 87, 573–582

32 Ramsey, J. (2006) Unreduced gametes and neopolyploids in natural populations of *Achillea borealis* (Asteraceae). *Heredity* 98, 143–150

33 Čertner, M. *et al.* (2017) Evolutionary dynamics of mixed-ploidy populations in an annual herb: dispersal, local persistence and recurrent origins of polyploids. *Ann. Bot.* 120, 303–315

34 Haan, A.D. *et al.* (1992) Production of 2n gametes in diploid subspecies of *Dactylis glomerata* L. 2. occurrence and frequency of 2n eggs. *Ann. Bot.* 69, 345–350

35 Bretagnolle, F. (2001) Pollen production and spontaneous polyploidization in diploid populations of *Anthoxanthum alpinum*. *Biol. J. Linn. Soc.* 72, 241–247

36 Maceira, N.O. *et al.* (1993) Competition between diploid and derivative autotetraploid *Dactylis glomerata* L. from Galicia. Implications for the establishment of novel polyploid populations. *New Phytol.* 124, 321–328

37 Kreiner, J.M. *et al.* (2017) Frequency and maintenance of unreduced gametes in natural plant populations: associations with reproductive mode, life history and genome size. *New Phytol.* 214, 879–889

38 Sonnleitner, M. *et al.* (2013) Parental ploidy strongly affects offspring fitness in heteroploid crosses among three cytotypes of autopolyploid *Jacobaea carniolica* (Asteraceae). *PLOS ONE* 8, e78959

39 Herben, T. *et al.* (2016) Reduced and unreduced gametes combine almost freely in a multiploidy system. *Perspect. Plant Ecol. Evol. Syst.* 18, 15–22

40 Baldwin, S.J. and Husband, B.C. (2011) Genome duplication and the evolution of conspecific pollen precedence. *Proc. R. Soc. Lond. B Biol. Sci.* 278, 2011–2017

41 Koutecký, P. *et al.* (2011) Hybridization between diploid *Centaurea pseudophrygia* and tetraploid C. jacea (Asteraceae): the role of mixed pollination, unreduced gametes, and mentor effects. *Biol. J. Linn. Soc.* 104, 93–106

42 Van de Peer, Y. *et al.* (2009) The flowering world: a tale of duplications. *Trends Plant Sci.* 14, 680–688

43 Levin, D.A. (1975) Minority cytotype exclusion in local plant populations. *Taxon*

44 Felber, F. (1991) Establishment of a tetraploid cytotype in a diploid population: Effect of relative fitness of the cytotypes. *J. Evol. Biol.* 4, 195–207

45 Kao, R.H. (2007) Asexuality and the coexistence of cytotypes. *New Phytol.* 175, 764–772

46 Husband, B.C. (2000) Constraints on polyploid evolution: a test of the minority cytotype exclusion principle. *Proc. R. Soc. Lond. B Biol. Sci.* 267, 217–223

47 Baack, E.J. (2005) Ecological factors influencing tetraploid establishment in snow buttercups (*Ranunculus adoneus* , Ranunculaceae): minority cytotype exclusion and barriers to triploid formation. *Am. J. Bot.* 92, 1827–1835

48 Husband, B.C. and Sabara, H.A. (2004) Reproductive isolation between autotetraploids and their diploid progenitors in fireweed, *Chamerion angustifolium* (Onagraceae). *New Phytol.* 161, 703–713

49 Fowler, N.L. and Levin, D.A. (1984) Ecological constraints on the establishment of a novel polyploid in competition with its diploid progenitor. *Am. Nat.* 124, 703–711

50 Rodriguez, D.J. (1996) A model for the establishment of polyploidy in plants. *Am. Nat.*

51 Hülber, K. *et al.* (2009) Ecological segregation drives fine-scale cytotype distribution of *Senecio carniolicus* in the Eastern Alps. *Preslia* 81, 309–319

52 Trávníček, P. *et al.* (2011) Remarkable coexistence of multiple cytotypes of the *Gymnadenia conopsea* aggregate (the fragrant orchid): evidence from flow cytometry. *Ann. Bot.* 107, 77–87

53 Trávníček, P. *et al.* (2011) Bridging global and microregional scales: ploidy distribution in *Pilosella echioides* (Asteraceae) in central Europe. *Ann. Bot.* 107, 443–454

54 Hanzl, M. *et al.* (2014) Nonadaptive processes governing early stages of polyploid evolution: Insights from a primary contact zone of relict serpentine *Knautia arvensis* (Caprifoliaceae). *Am. J. Bot.* 101, 935–945

55 Baack, E.J. and Stanton, M.L. (2005) Ecological factors influencing tetraploid speciation in snow buttercups (*Ranunculus adoneus*): niche differentiation and tetraploid establishment. *Evolution* 59, 1936–1944

56 Martin, S.L. and Husband, B.C. (2012) Whole genome duplication affects evolvability of flowering time in an autotetraploid plant. *PLOS ONE* 7, e44784

57 Sonnleitner, M. *et al.* (2016) Ecological differentiation of diploid and polyploid cytotypes of *Senecio carniolicus* sensu lato (Asteraceae) is stronger in areas of sympatry. *Ann. Bot.* 117, 269–276

58 Cosendai, A.-C. *et al.* (2013) Geographical parthenogenesis and population genetic structure in the alpine species *Ranunculus kuepferi* (Ranunculaceae). *Heredity* 110, 560–569

59 Kao, R.H. and Parker, I.M. (2010) Coexisting cytotypes of *Arnica cordifolia*: morphological differentiation and local-scale distribution. *Int. J. Plant Sci.* 171, 81–89

60 Baldwin, S.J. and Husband, B.C. (2013) The association between polyploidy and clonal reproduction in diploid and tetraploid *Chamerion angustifolium*. *Mol. Ecol.* 22, 1806–1819

61 Buggs, R.J.A. and Pannell, J.R. (2007) Ecological differentiation and diploid superiority across a moving ploidy contact zone. *Evolution* 61, 125–140

62 Lumaret, R. *et al.* (1987) Polyploidy and habitat differentiation in *Dactylis glomerata* L. from Galicia (Spain). *Oecologia* 73, 436–446

63 Ramsey, J. (2011) Polyploidy and ecological adaptation in wild yarrow. *Proc. Natl. Acad. Sci.* 108, 7096–7101

64 Duchoslav, M. *et al.* (2016) The ecological performance of tetra-, penta- and hexaploid geophyte *Allium oleraceum* in reciprocal transplant experiment may explain the occurrence of multiple-cytotype populations. *J. Plant Ecol.* DOI: 10.1093/jpe/rtw039

65 Laport, R.G. *et al.* (2013) Ecological niche modeling implicates climatic adaptation, competitive exclusion, and niche conservatism among *Larrea tridentata* cytotypes in North American deserts. *J. Torrey Bot. Soc.* 140, 349–363

66 Glennon, K.L. *et al.* (2014) Evidence for shared broad-scale climatic niches of diploid and polyploid plants. *Ecol. Lett.* 17, 574–582

67 Richardson, M.L. and Hanks, L.M. (2011) Differences in spatial distribution, morphology, and communities of herbivorous insects among three cytotypes of *Solidago altissima* (Asteraceae). *Am. J. Bot.* 98, 1595–1601

68 Kirchheimer, B. *et al.* (2016) A matter of scale: apparent niche differentiation of diploid and tetraploid plants may depend on extent and grain of analysis. *J. Biogeogr.* 43, 716–726

69 Jersáková, J. *et al.* (2010) Absence of pollinator-mediated premating barriers in mixed-ploidy populations of *Gymnadenia conopsea* s.l. (Orchidaceae). *Evol. Ecol.* 24, 1199–1218

70 Kennedy, B.F. *et al.* (2006) Pollinator-mediated assortative mating in mixed ploidy populations of *Chamerion angustifolium* (Onagraceae). *Oecologia* 150, 398–408

71 Castro, S. *et al.* (2011) Breeding barriers at a diploid–hexaploid contact zone in *Aster amellus*. *Evol. Ecol.* 25, 795–814

72 Gross, K. and Schiestl, F.P. (2015) Are tetraploids more successful? Floral signals, reproductive success and floral isolation in mixed-ploidy populations of a terrestrial orchid. *Ann. Bot.* 115, 263–273

73 Barringer, B.C. (2007) Polyploidy and self-fertilization in flowering plants. *Am. J. Bot.* 94, 1527–1533

74 Husband, B.C. *et al.* (2008) Mating consequences of polyploid evolution in flowering plants: current trends and insights from synthetic polyploids. *Int. J. Plant Sci.* 169, 195–206

75 Husband, B.C. *et al.* (2016) Direct vs. indirect effects of whole-genome duplication on prezygotic isolation in *Chamerion angustifolium*: Implications for rapid speciation. *Am. J. Bot.* 103, 1259–1271

76 Segraves, K.A. and Anneberg, T.J. (2016) Species interactions and plant polyploidy. *Am. J. Bot.* 103, 1326–1335

77 Bretagnolle, F. and Thompson, J.D. (2001) Phenotypic plasticity in sympatric diploid and autotetraploid *Dactylis glomerata*. *Int. J. Plant Sci.* 162, 309–316

78 Hersh, E. *et al.* (2016) Attack of the clones: reproductive interference between sexuals and asexuals in the *Crepis* agamic complex. *Ecol. Evol.* 6, 6473–6483

79 Kliber, A. *et al.* (2005) Interaction between founder effect and selection during biological invasion in an aquatic plant. *Evolution* 59, 1900–1913

80 Duchoslav, M. *et al.* (2010) Complex distribution patterns, ecology and coexistence of ploidy levels of *Allium oleraceum* (Alliaceae) in the Czech Republic. *Ann. Bot.* 105, 719–735

81 Krahulcová, A. *et al.* (2009) Enriching ploidy level diversity: the role of apomictic and sexual biotypes of *Hieracium* subgen. *Pilosella* (Asteraceae) that coexist in polyploid populations. *Folia Geobot.* 44, 281–306

82 Mráz, P. *et al.* (2008) Cytogeography of *Pilosella officinarum* (Compositae): Altitudinal and longitudinal differences in ploidy level distribution in the Czech Republic and Slovakia and the general pattern in Europe. *Ann. Bot.* 101, 59–71

83 Thompson, K.A. *et al.* (2014) Climatic niche differences between diploid and tetraploid cytotypes of *Chamerion angustifolium* (Onagraceae). *Am. J. Bot.* 101, 1868–1875

84 Münzbergová, Z. (2007) No effect of ploidy level in plant response to competition in a common garden experiment. *Biol. J. Linn. Soc.* 92, 211–219

85 Roxburgh, S.H. *et al.* (2004) The intermediate disturbance hypothesis: patch dynamics and mechanisms of species coexistence. *Ecology* 85, 359–371

86 Meirmans, P.G. *et al.* (1999) Anthropogenic disturbance and habitat differentiation between sexual diploid and apomictic triploid *Taraxacum* sect. *Ruderalia*. *Folia Geobot.* 34, 451–469

87 Felber, F. and Bever, J.D. (1997) Effect of triploid fitness on the coexistence of diploids and tetraploids. *Biol. J. Linn. Soc.* 60, 95–106

88 Kron, P. and Husband, B.C. (2009) Hybridization and the reproductive pathways mediating gene flow between native *Malus coronaria* and domestic apple, *M. domestica*. *Botany* 87, 864–874

89 Keeler, K.H. (2004) Impact of intraspecific polyploidy in *Andropogon gerardii* (Poaceae) populations. *Am. Midl. Nat.* 152, 63–74

90 Dijk, P.V. *et al.* (1992) Single cytotype areas in autopolyploid *Plantago media* L. *Biol. J. Linn. Soc.* 46, 315–331

91 Ayres, D.R. and Strong, D.R. (2001) Origin and genetic diversity of *Spartina anglica* (Poaceae) using nuclear DNA markers. *Am. J. Bot.* 88, 1863–1867

92 Soltis, D.E. *et al.* (2004) Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic, genomic and genetic comparisons. *Biol. J. Linn. Soc.* 82, 485–501

93 Vallejo-Marin, M. (2012) *Mimulus peregrinus* (Phrymaceae): A new British allopolyploid species. *PhytoKeys* 14, 1–14

94 Abbott, R.J. and Lowe, A.J. (2004) Origins, establishment and evolution of new polyploid species: *Senecio cambrensis* and *S. eboracensis* in the British Isles. *Biol. J. Linn. Soc.* 82, 467–474

95 Petit, M. *et al.* (2010) Mobilization of retrotransposons in synthetic allotetraploid tobacco. *New Phytol.* 186, 135–147

96 Szadkowski, E. *et al.* (2011) Polyploid formation pathways have an impact on genetic rearrangements in resynthesized *Brassica napus*. *New Phytol.* 191, 884–894

97 Oswald, B.P. and Nuismer, S.L. (2011) Neopolyploidy and diversification in *Heuchera grossulariifolia*. *Evolution* 65, 1667–1679

98 Yant, L. *et al.* (2013) Meiotic adaptation to genome duplication in *Arabidopsis arenosa*. *Curr. Biol.* 23, 2151–2156

99 Zhang, H. *et al.* (2013) Intrinsic karyotype stability and gene copy number variations may have laid the foundation for tetraploid wheat formation. *Proc. Natl. Acad. Sci.* 110, 19466–19471

100 Samans, B. *et al.* (2017) Surviving a genome collision: genomic signatures of allopolyploidization in the recent crop species. *Plant Genome* Published online August 10, 2017. http://dx.doi.org/10.3835/plantgenome2017.02.0013

101 Petit, C. *et al.* (1997) Variation in flowering phenology and selfing rate across a contact zone between diploid and tetraploid *Arrhenatherum elatius* (Poaceae). *Heredity* 79, 31–40

102 Nuismer, S.L. and Cunningham, B.M. (2005) Selection for phenotypic divergence between diploid and autotetraploid *Heuchera grossulariifolia*. *Evolution* 59, 1928–1935

103 Suda, J. and Herben, T. (2013) Ploidy frequencies in plants with ploidy heterogeneity: fitting a general gametic model to empirical population data. *Proc. R. Soc. B Biol. Sci.* 280, 20122387

Supplementary table S1. Details on the 69 reviewed mixed ploidy species.

Species	Family	Growth form	Type of polypl.	Asexuality*	Major / rare cytotypes	Ploidy combinations in natural populations	Max # of cytotypes per pop
Acacia senegal	Fabaceae	tree	-	none	2,4 (3,6)	2+3,2+4,2+6	2
Achillea borealis	Asteraceae	perennial herb	auto	some (cl.)	4,6	4+6	2
Actinidia chinensis	Actinidiaceae	liana	-	none	2,4,6 (5)	2+4,2+6,4+6,4+5+6	3
Allium oleraceum	Amaryllidaceae	perennial herb	auto	strong (bl.)	4,5,6 (3)	4+5, 4+6, 5+6, 4+5+6	3
Allium przewalskianum	Amaryllidaceae	perennial herb	-	none	2,4	2+4	2
Alnus glutinosa	Betulaceae	tree	auto	none	2,4 (3)	2+3, 2+4, 2+3+4, 3+4	3
Alyssum montanum	Brassicaceae	perennial herb	auto	none	2,4 (3)	2+3	2
Andropogon gerardii	Poaceae	perennial herb	auto	some (cl.)	6,9 (7 or 8)	6+9, 6+7/8?, 6+7/8?+9	2
Arabidopsis arenosa	Brassicaceae	perennial herb	auto	none	2,4 (3)	2+3, 2+4	2
Arnica cordifolia	Asteraceae	perennial herb	-	strong (cl., ag.)	(2),3,4 (5)	3+4, 3+4+5, 3+4+6 (6 only in seeds)	3
Arrhenatherum elatius	Poaceae	perennial herb	auto	some (cl.)	2,4 (3)	2+3+4	3
Artemisia sieberi	Asteraceae	perennial herb	-	none	2,4	-	1
Artemisia tridentata	Asteraceae	shrub	auto	some (cl.)	2,4 (6)	2+4, 2+6	2
Aster amellus	Asteraceae	perennial herb	auto	none	2,6 (3,5,7,8,9)	2+3, 2+6, 6+7, 6+8, 6+7+8, 6+7+8+9	4
Aucuba japonica	Garryaceae	shrub	-	none	2,4	-	1
Brachypodium distachyon	Poaceae	perennial herb	allo	none	2,4	2+4	2
Butomus umbellatus	Butomaceae	perennial herb	auto	strong (r.b., bl.)	2,3	-	1
Cardamine amara	Brassicaceae	perennial herb	auto	some (cl.)	2,4 (3)	2+4, 2+3, 2+3+4	3
Cardamine torrentis s.l.	Brassicaceae	perennial herb	-	none	4,8 (6)	4+6	2
Cardamine yezoensis	Brassicaceae	perennial herb	-	none	6 (8,9,10,11,12)	6+8+9, 6+9+10, 6+10, 6+11,6+8+9+11,9+10+11,9+11	4
Centaurea jacea agg.	Asteraceae	perennial herb	auto	none	2,4 (6)	2+4, 4+6	2
Centaurea phrygia	Asteraceae	perennial herb	auto	none	2,4 (3,5,6)	2+3, 2+4, 2+3+4, 2+4+5, 4+5, 4+6	3
Centaurea stoebe	Asteraceae	perennial herb	allo	none	2,4 (3)	2+4, 2+3+4	3

* cl. = clonality, bl. = bulbils, ag. = agamospermy, r.b. = rhizome buds

Supplementary table S1 (continued).

Species	Family	Growth form	Type of polypl.	Asexuality*	Major / rare cytotypes	Ploidy combinations in natural populations	Max # of cytotypes per pop
Chamerion angustifolium	Onagraceae	perennial herb	auto	some (cl.)	2,4 (3,5)	2+3, 2+4, 3+4, 2+3+4, 2+4+5, 4+5	3
Dactylis glomerata	Poaceae	perennial herb	auto	some (cl.)	2,4	2+4	2
Dactylorhiza maculata s.l.	Orchidaceae	perennial herb	auto	none	2,4 (3)	2+3+4	3
Deschampsia cespitosa	Poaceae	perennial herb	auto	some (cl.)	2,4	2+4	2
Dianthus broteri	Caryophyllaceae	perennial herb	-	none	2,4,6,12 (3)	2+3	2
Empetrum nigrum s.l.	Ericaceae	shrub	-	some (cl.)	2,4 (3)	2+3, 2+4, 2+3+4, 3+4	3
Galax urceolata	Diapensiaceae	perennial herb	auto	none	2,4 (3)	2+3, 2+4, 3+4, 2+3+4	3
Galium valdepilosum	Rubiaceae	perennial herb	-	none	2,4	-	1
Gymnadenia conopsea	Orchidaceae	perennial herb	auto	none	2,4 (3,5,6)	2+3, 2+4, 3+4, 4+5, 4+6, 2+3+4, 2+3+6, 2+4+5, 2+4+6, 4+5+6, 2+3+4+5, 2+3+4+6, 3+4+5+6, 2+3+4+5+6	5
Heuchera cylindrica	Saxifragaceae	perennial herb	auto	none	2,4	-	1
Heuchera grossulariifolia	Saxifragaceae	perennial herb	auto	some (cl.)	2,4 (3)	2+4, 2+3, 2+3+4	3
Hypochaeris incana	Asteraceae	perennial herb	auto	none	2,4	2+4	2
Ixeris nakazonei	Asteraceae	perennial herb	allo	none	4,6,8 (3,5,7)	nearly all possible combinations	4
Jacobaea vulgaris	Asteraceae	perennial herb	-	none	4,8 (6)	4+6, 4+8, 4+6+8	3
Knautia arvensis	Caprifoliaceae	perennial herb	auto	some (cl.)	2,4 (3)	2+3, 2+4, 2+4+6	3
Larrea tridentata	Zygophyllaceae	shrub	auto	none	2,4,6 (3,5)	2+4, 4+6, 4+5+6, 2+3	3
Lilium lancifolium	Liliaceae	perennial herb	-	some (bl.)	2,3	2+3	1
Lippia alba	Verbenaceae	shrub	-	none	2,4 (3,6)	-	1
Lotus alpinus	Fabaceae	perennial herb	auto	some (cl.)	2,4	2+4	2
Lythrum salicaria	Lythraceae	perennial herb	-	none	2,4 (3, 6)	3+4, 4+6	2
Melampodium cinereum	Asteraceae	perennial herb	auto	some (cl.)	2,4 (3)	2+3, 2+4	2
Melampodium leucanthum	Asteraceae	perennial herb	auto	some (cl.)	2,4 (3)	2+4, 2+3+4	2
Mercurialis annua	Euphorbiaceae	annual herb	-	none	2,6 (4,8)	-	1

* cl. = clonality, bl. = bulbils

Supplementary table S1 (continued).

Species	Family	Growth form	Type of polypl.	Asexuality*	Major / rare cytotypes	Ploidy combinations in natural populations	Max # of cytotypes per pop
Odontites vernus	Orobanchaceae	annual herb	-	none	2,4	2+4	2
Oxalis obtusa	Oxalidaceae	perennial herb	auto	none	2,4,6 (3,5,7,8)	2+4, 2+6, 4+6, 2+3, 2+5, 2+8, 3+6	2
Oxalis pes-caprae	Oxalidaceae	perennial herb	auto	strong (bl.)	2,4,5 (3)	4+5	2
Parasenecio auriculata	Asteraceae	perennial herb	auto	none	2,4 (3)	2+3, 2+4	2
Phleum pratense	Poaceae	perennial herb	-	none	2,6	2+6	2
Pilosella echioides	Asteraceae	perennial herb	auto	some (cl.?) / strong (ag.)	2,4 (3,5,6)	2+3,2+3+4, 2+3+4+5, 2+3+4+5+6, 3+4+5, 4+6, 4+5+6	5
Pilosellla rhodopea	Asteraceae	perennial herb	auto	none	2,3,4 (5)	2+3,2+3+4, 2+3+4+5,3+4, 3+4+5	4
Plantago media	Plantaginaceae	perennial herb	auto	none	2,4 (3)	2+4, 2+3	2
Populus tremuloides	Salicaceae	tree	auto	strong (cl.)	2,3	2+3	2
Ranunculus adoneus	Ranunculaceae	perennial herb	auto	none	2,4 (3,6)	2+4, 2+3+4, 4+6	3
Ranunculus kuepferi	Ranunculaceae	perennial herb	auto	strong (ag.)	2,4 (3,5,6)	2+3, 4+6, 3+4, 2+3+4, 3+4+5, 2+3+4+5	4
Saxifraga oppositifolia	Saxifragaceae	perennial herb	auto	some (cl.)	2,4 (3)	2+3, 2+4, 2+3+4	3
Senecio carniolicus	Asteraceae	perennial herb	auto	none	2,4,6 (3,5,7,8,9)	2+3, 2+4, 2+6, 4+5, 4+6, 6+7, 6+9, 2+4+5, 2+4+6, 2+5+6, 2+6+7, 2+6+9, 4+5+6, 6+7+9, 2+4+5+6, 2+4+6+7, 2+6+8+9, 2+3+4+5+6	5
Solidago altissima	Asteraceae	perennial herb	auto	some (cl.)	2,4,6	2+4, 2+6, 4+6, 2+4+6	3
Solidago gigantea	Asteraceae	perennial herb	-	some (cl.)	2,4,6 (3, 5)	2+4, 3+4+5	3
Spartina pectinata	Poaceae	perennial herb	-	some (cl.)	4,8 (6)	4+6	2
Spergularia echinosperma	Caryophyllaceae	annual herb	-	none	2,4	2+4	2
Symphytum tuberosum	Boraginaceae	perennial herb	`-	none	4,12 (6,10,14)	4+12,4+6,10+12,12+14	2
Taraxacum sect *Taraxacum*	Asteraceae	perennial herb	-	strong (ag.)	2,3 (4)	2+3, 2+3+4	3
Tripleurospermum inodorum	Asteraceae	annual herb	?auto	none	2,4 (3,5,6)	2+4, 2+3, 3+4, 2+3+4, 2+4+5, 4+6	3
Turnera sidoides pinnatifida	Turneraceae	perennial herb	auto	none	2,4 (3, 6)	2+3	2
Vaccinium oxycoccos	Ericaceae	perennial herb	auto	some (cl.)	4,6 (5)	4+6, 4+5, 5+6, 4+5+6	3
Vicia cracca	Fabaceae	perennial herb	auto	none	2,4 (3)	2+3, 2+4	2

* cl. = clonality, bl. = bulbils, ag. = agamospermy,

Supplementary table S1 (continued).

Species	% secondary / intermediate cytotypes	% of mixed-ploidy populations#	Type of contact zone*	Geography of contact zone	Between-ploidy gene flow	Intermediate cytotypes are hybrids?	Intrapop recur. form. of polyploid	Molecular markers used for study of gene flow
Acacia senegal	2.5/2	9/5.6/3.7/3.7	-	sympatric	-	-	-	-
Achillea borealis	- / -	-	sec.	parapatric	-	-	y	-
Actinidia chinensis	0.6/0.6	-	sec.	parapatric	y (low)	(y)	-	-
Allium oleraceum	0.06/-	21.5/21.5/0/0	sec. (+?prim.)	sympatric	y	y	y	allozymes
Allium przewalskianum	- / -	6.5/6.5/ - / -	-	sympatric	-	-	-	-
Alnus glutinosa	0.2/0.2	2.4/1.4/1/2.4	sec.	parapatric	y (low)	y / n	-	SSR
Alyssum montanum	0.2/0.2	1.8/0/1.8/1.8	sec.	mosaic	n	n	-	AFLP
Andropogon gerardii	- / -	46.9/46.9/ - / -	-	sympatric	(y)	(y)	y	-
Arabidopsis arenosa	0.2/0.2	4.1/0.5/3.1/3.1	sec.+prim.	parapatric	y	n	-	RADseq
Arnica cordifolia	1.3/-	-	-	sympatric	y (low)	-	y	plastid DNA sequences
Arrhenatherum elatius	- / -	-	sec.	parapatric	n	y	n	allozymes
Artemisia sieberi	- / -	0/0/ - / -	-	parapatric	-	-	-	-
Artemisia tridentata	0.1/ -	-	sec.	sympatric	-	-	-	-
Aster amellus	0.4/-	9.5/0.3/9.2/9.2	sec.	mosaic	n	-	(n)	SSR
Aucuba japonica	- / -	-	-	mosaic	-	-	-	-
Brachypodium distachyon	- / -	21/21/ - / -	sec.	parapatric	n	-	-	-
Butomus umbellatus	- / -	-	sec.	mosaic	y (low)	-	y	RAPD, allozymes
Cardamine amara	2.5/2.5	6/5/0.7/1	sec.	parapatric	y	y	-	SSR, AFLP
Cardamine torrentis s.l.	0.9/0.9	5/0/5/5	-	allopatric	-	-	-	-
Cardamine yezoensis	22.3/ -	34.8/0/34.8/34.8	-	-	-	-	-	-
Centaurea jacea agg.	0.2/ -	-	sec.	parapatric	n	-	-	allozymes
Centaurea phrygia	0.04/0.03	-	sec.	parapatric	y (low)	(y)	y	-
Centaurea stoebe	0.04/0.04	3.5/3.5/0/1	sec.	mosaic	n	y	-	plastid and ITS sequences, SSR

#All mixed / dominant ploidy mixed / one dominant+rare ploidy mixed / mixed involving rare cytotype.

* prim. = primary, sec. = secondary

Supplementary table S1 (continued).

Species	% secondary / intermediate cytotypes	% of mixed-ploidy populations[#]	Type of contact zone*	Geography of contact zone	Between-ploidy gene flow	Intermediate cytotypes are hybrids?	Intrapop recur. form. of polyploid	Molecular markers used for study of gene flow
Chamerion angustifolium	2.2/2.1	-	sec.	parapatric	y	-	y	-
Dactylis glomerata	-/-	-	sec.+prim.	sympatric	-	-	-	-
Dactylorhiza maculata s.l.	10.5/10.5	-	sec.	mosaic	y (low)	y	-	nuclear and plastid DNA sequences
Deschampsia cespitosa	- / -	-	-	sympatric	-	-	-	-
Dianthus broteri	2/2	4/0/4/4	sec.	mosaic	-	-	-	-
Empetrum nigrum s.l.	1.9/1.9	-	sec.	parapatric	-	(y)	-	-
Galax urceolata	11/11	-	-	parapatric	-	-	-	SSR
Galium valdepilosum	- / -	0/0/ - / -	sec.	parapatric	-	-	-	-
Gymnadenia conopsea	2.1/1.6	42.9/15.8/27.1/39.7	-	sympatric	-	-	-	-
Heuchera cylindrica	- / -	0/0/ - / -	-	parapatric	-	-	-	-
Heuchera grossulariifolia	1.4/1.4	20.1/17.2/2.9/6.9	sec.	sympatric	y (low)	(y)	y	plastid DNA sequences
Hypochaeris incana	- / -	3.2/3.2/ - / -	-	mosaic	-	-	y	-
Ixeris nakazonei	3.2/3.2	15.5/12.4/3.1/9.3	-	sympatric	y	(y)	-	-
Jacobaea vulgaris	1.4/1.4	11.8/10.5/1.3/5.3	-	mosaic	n	(y)	-	-
Knautia arvensis	0.04/0.04	6.2/5.5/0.7/0.7	sec.+prim.	mosaic	n	(n)	-	AFLP
Larrea tridentata	0.3/0.3	6.3/6.3/0/3.6	sec.	parapatric	y	y	y	plastid DNA, AFLP
Lilium lancifolium	- / -	1.5/1.5/ - / -	-	parapatric	-	-	-	-
Lippia alba	17/15.1	-	-	sympatric	-	(y)	-	-
Lotus alpinus	- / -	-	sec.	parapatric	n	-	n	allozymes
Lythrum salicaria	9.3/0.9	3.2/0/3.2/3.2	-	allopatric	-	-	-	-
Melampodium cinereum	0.6/0.6	-	sec.(+prim.?)	parapatric	-	n	(y)	-
Melampodium leucanthum	0.2/0.2	-	sec.(+prim.?)	parapatric	-	n	(y)	-
Mercurialis annua	-/-	-	sec.	parapatric	-	-	-	-

[#] All mixed / dominant ploidy mixed / one dominant+rare ploidy mixed / mixed involving rare cytotype.

* prim. = primary, sec. = secondary

Supplementary table S1 (continued).

Species	% secondary / intermediate cytotypes	% of mixed-ploidy populations[#]	Type of contact zone*	Geography of contact zone	Between-ploidy gene flow	Intermediate cytotypes are hybrids?	Intrapop recur. form. of polyploid	Molecular markers used for study of gene flow
Odontites vernus	- / -	-	-	sympatric	-	-	-	-
Oxalis obtusa	7.3/2	-	sec.	sympatric	-	(y)(5x,7x)	-	-
Oxalis pes-caprae	4.8/4.8	30.8/30.8/ - / -	sec.	sympatric	-	-	(n)	-
Parasenecio auriculata	0.4/0.4	6.1/6.1/0/3	-	mosaic	-	-	-	-
Phleum pratense	- / -	22/22/ - / -	sec.	sympatric	n	-	-	-
Pilosella echioides	7.9/5.5*	23.9/8.7/15.2/23.9	sec.+?prim.	mosaic	y	y	y	-
Pilosellla rhodopea	2.4/49.2	75/75/0/16.7	prim.	sympatric	y	y	-	nuclear and plastid DNA sequences, allozymes
Plantago media	-/-	-	sec.	parapatric	n	(n)	y	plastid DNA sequences
Populus tremuloides	-/ -	64.3/64.3/ - / -	-	sympatric	-	-	-	-
Ranunculus adoneus	1.3/1.2	10.5/2.6/7.9/7.9	sec.	parapatric	-	(y)	y	-
Ranunculus kuepferi	7.6/6	15.3/5.1/10.2/13.6	sec.	parapatric	n	(y)	-	-
Saxifraga oppositifolia	4.1/4.1	-	sec.	sympatric	-	y	-	AFLP
Senecio carniolicus	1.1/0.9	56/45/14/25	sec.	sympatric	y (low)	y(5x)/n(3x)	n	AFLP
Solidago altissima	-/-	-	?sec.	parapatric	-	(y)	y	-
Solidago gigantea	0.2/0.2	-	-	mosaic	-	y	-	-
Spartina pectinata	1.1/ -	-	sec.+prim.	parapatric	-	n	-	-
Spergularia echinosperma	- / -	14/14/ - / -	-	sympatric	n	-	-	nuclear and plastid DNA sequences
Symphytum tuberosum	3.1/ -	9.2/1.5/7.7/7.7	sec.	parapatric	-	-	-	-
Taraxacum sect *Taraxacum*	0.03/ -	-	-	sympatric	-	-	-	-
Tripleurospermum inodorum	1.5/1.4	-	sec.	sympatric	y (low)	y	y	-
Turnera sidoides pinnatifida	- / -	7.7/0/7.7/7.7	prim.	sympatric	-	-	-	-
Vaccinium oxycoccos	27.4/27.4	-	-	mosaic	-	(y)	-	-
Vicia cracca	0.1/0.1	7/6.2/0.8/0.8	sec.+prim.	parapatric	n	n	n	allozymes

[#] All mixed / dominant ploidy mixed / one dominant+rare ploidy mixed / mixed involving rare cytotype.

* prim. = primary, sec. = secondary

Supplementary table S1 (continued).

Species	Spatial segreg. large-scale	Spatial segreg. intrapop	Altitudinal segregation	Colonization history	Habitat segregation	Compe-tition	Dispersal limitation	Phenology shift	Pollinator fidelity	Other interactions	Breeding/mating system shift
Acacia senegal	y	-	-	(y)	-	-	-	-	-	-	-
Achillea borealis	y	-	-	-	y	-	(y)	y	-	-	-
Actinidia chinensis	y	y	y	-	(n)	-	-	(y)	-	-	-
Allium oleraceum	n	y	n	(y)	y	n	(y)	-	-	-	y
Allium przewalskianum	y	-	(y)	(y)	-	-	-	-	-	-	-
Alnus glutinosa	y	-	-	y	(y)	-	-	-	-	-	-
Alyssum montanum	y	-	-	-	n	-	-	-	-	-	-
Andropogon gerardii	y	y	-	(y)	y	(y)	-	-	-	-	-
Arabidopsis arenosa	y	-	n	y	n	-	(y)	-	-	-	-
Arnica cordifolia	y	y	-	n	n	y	(n)	y	-	-	-
Arrhenatherum elatius	y	y	y	(y)	(y)	y	-	y	-	-	-
Artemisia sieberi	y	-	n	-	y	-	-	-	-	-	-
Artemisia tridentata	y	y	-	-	y	(y)	-	-	-	-	-
Aster amellus	y	n	-	-	n	n	-	n	n	y (herbiv.)	y
Aucuba japonica	y		-	(y)	-	-	-	-	-	-	-
Brachypodium distachyon	y	-	y	(y)	y	-	(y)	n	-	-	-
Butomus umbellatus	y	-	n	-	y	y	(n)	-	-	-	y
Cardamine amara	y	y	y	(y)	y	(y)	(y)	-	-	-	n
Cardamine torrentis s.l.	y	-	-	-	-	-	-	-	-	-	-
Cardamine yezoensis	n	-	n	-	-	-	-	-	-	-	-
Centaurea jacea agg.	y	-	y	-	n	-	-	(n)	-	-	(n)
Centaurea phrygia	y	-	-	-	-	-	-	(y)	-	y (herbiv.)	n
Centaurea stoebe	y	y	(n)	y	y	(y)	(y)	n	(n)	n (herbiv.)	n

Supplementary table S1 (continued).

Species	Spatial segreg. large-scale	Spatial segreg. intrapop	Altitudinal segregation	Colonization history	Habitat segregation	Compe-tition	Dispersal limitation	Phenology shift	Pollinator fidelity	Other interactions	Breeding/mating system shift
Chamerion angustifolium	y	y	y	y	y	n	(n)	y	y	-	y
Dactylis glomerata	n	y	(n)	(n)	y	y	-	y	-	-	-
Dactylorhiza maculata s.l.	y	y	-	-	y	-	(n)	-	-	-	-
Deschampsia cespitosa	y	-	n	y	y	-	-	-	-	-	-
Dianthus broteri	y	-	-	-	-	-	-	-	-	-	-
Empetrum nigrum s.l.	y	y	-	-	y	-	-	-	-	-	y
Galax urceolata	y		-	-	-	-	-	-	-	-	-
Galium valdepilosum	y	-	n	-	y	-	(y)	-	-	-	-
Gymnadenia conopsea	n	y	y	-	-	-	(n)	y / n	n	y (mycorh.)	n
Heuchera cylindrica	y		-	(y)	n	-	(y)	-	-	-	-
Heuchera grossulariifolia	y	-	(n)	(y)	(n)	-	-	y	y	y (herbiv.)	-
Hypochaeris incana	y		-	y	-	-	-	-	-	-	-
Ixeris nakazonei	y	-	-	-	-	-	-	-	-	-	-
Jacobaea vulgaris	y	-	-	y	-	-	-	(n)	-	-	-
Knautia arvensis	y	y	y	y	n	(n)	(y)	-	-	-	-
Larrea tridentata	y	y	-	y	y	-	-	y	-	-	-
Lilium lancifolium	y	-	-	-	(y)	-	-	-	-	-	-
Lippia alba	-	-	-	-	-	-	-	-	-	-	-
Lotus alpinus	y	y	y	-	-	(y)	-	y	-	-	-
Lythrum salicaria	y	-	-	-	-	-	-	-	-	-	-
Melampodium cinereum	y	-	-	-	-	-	-	-	-	-	-
Melampodium leucanthum	y	-	-	-	-	-	-	-	-	-	-
Mercurialis annua	n	-	-	(y)	n	y	-	-	-	-	y

Supplementary table S1 (continued).

Species	Spatial segreg. large-scale	Spatial segreg. intrapop	Altitudinal segregation	Colonization history	Habitat segregation	Compe-tition	Dispersal limitation	Phenology shift	Pollinator fidelity	Other interactions	Breeding/mating system shift
Odontites vernus	y	-	-	-	-	-	-	y	-	-	-
Oxalis obtusa	y	y	n	-	y	-	-	-	-	-	-
Oxalis pes-caprae	n	-	-	-	y	-	-	-	-	-	y
Parasenecio auriculata	y		n	-	y	-	-	-	-	-	-
Phleum pratense	n	-	-	(y)	-	-	-	-	-	-	-
Pilosella echioides	y	y	(n)	n	(y)	-	(y)	(n)	(n)	-	y / n
Pilosellla rhodopea	n	-	-	-	-	-	-	-	-	-	-
Plantago media	y	-	(y)	y	(n)	-	(y)	y	-	-	-
Populus tremuloides	n	-	-	-	-	-	-	-	-	-	y
Ranunculus adoneus	y	y	(n)	(y)	n	(n)	y	(n)	-	-	-
Ranunculus kuepferi	y	-	y	y	y	-	-	-	-	-	y
Saxifraga oppositifolia	n	n	-	(y)	y	(y)	-	-	-	-	-
Senecio carniolicus	y	y	y	y	y	(y)	(y)	(n)	-	-	n
Solidago altissima	y	n/(y)	-	-	y	n	(n)	(y)	-	n (herbiv.)	-
Solidago gigantea	y	-	-	-	-	-	(n)	-	-	-	-
Spartina pectinata	y	y	-	-	-	(y)	-	y	-	-	-
Spergularia echinosperma	-	-	-	-	-	-	-	-	-	-	-
Symphytum tuberosum	y	-	y	(y)	(y)	-	-	-	-	-	-
Taraxacum sect *Taraxacum*	y	n	y	-	y	-	-	-	-	-	y
Tripleurospermum inodorum	y	y	(n)	-	(n)	-	(y)	(n)	-	-	n
Turnera sidoides pinnatifida	y	y	-	-	y / n	-	-	-	-	-	-
Vaccinium oxycoccos	n	n	n	-	n	-	-	(n)	-	-	n
Vicia cracca	y	y	n	-	-	(y)	-	-	-	-	n

Supplementary table S1 (continued).

Species	Siring success	Seed abortion	Low hybrid fitness	Overall clonality	Use of neopoly-ploids	Other reason for coexistence*	References
Acacia senegal	-	-	-	-	-		Odee et al. (2015)
Achillea borealis	-	-	(y)	-	y (natural)		Ramsey 2007, 2011, Ramsey et al. 2008
Actinidia chinensis	-	-	y	-	-		Li et al. (2010); Liu et al. (2015)
Allium oleraceum	-	-	-	y	-	s. seed bank	Duchoslav et al. (2010, 2017); Šafářová and Duchoslav (2010); Šafářová et al. (2011); Fialová et al. (2014); Fialová and Duchoslav (2014); Duchoslav and Staňková (2015)
Allium przewalskianum	-	-	-	-	-		Xie Kui et al. (2008)
Alnus glutinosa	-	-	-	-	-		Mandak et al. (2015)
Alyssum montanum	-	-	-	-	-		Španiel et al. (2011)
Andropogon gerardii	-	y	n	(y)	-	longev.	Keeler (1992, 2004); Keeler & Davis (1999); McAllister et al. (2015)
Arabidopsis arenosa	-	-	-	-	-		Kolář et al. (2015); Arnold et al. (2015)
Arnica cordifolia	-	-	-	y	-	agam.	Kao (2007, 2008); Kao Parker (2010)
Arrhenatherum elatius	-	-	y	(y)	-		Petit and Thompson (1997); Petit et al. (1997)
Artemisia sieberi	-	-	-	-	-		Jalili et al. (2013)
Artemisia tridentata	-	y	-	-	-	veg. repr., longev.	McArthur and Sanderson (1999)
Aster amellus	-	y	y	-	-		Munzbergová (2006, 2007); Mandáková and Munzbergová (2006, 2008); Raabová et al. (2008); Castro et al. (2011, 2012); Munzbergová et al. (2013)
Aucuba japonica	-	-	-	-	-		Ohi et al. (2003)
Brachypodium distachyon	-	-	-	-	-	4x better water use efficiency	Manzaneda et al. (2012)
Butomus umbellatus	-	(y)	(y)	y	-	disturbance	Hroudová & Zákravský (1993a, 1993b); Krahulcová & Jarolímová (1993); Kirschner et al. (2004); Kliber & Eckert (2005)
Cardamine amara	-	-	-	y	-	veg. repr.	Zozomová-Lihová et al. (2015)
Cardamine torrentis s.l.	-	-	-	-	-		Marhold et al. (2010)
Cardamine yezoensis	-	-	-	-	-		Marhold et al. (2010)
Centaurea jacea agg.	-	y	-	-	-		Hardy et al. (2000)
Centaurea phrygia	-	y	(y)	-	-		Koutecký et al. (2012); Munzbergová et al. (2015)
Centaurea stoebe	-	y	y	-	-	recent spread of 4x	Španiel et al (2008); Treier et al. (2009); Collins et al. (2011); Mráz et al. (2012); Hahn et al. (2012); Otisková et al. (2014)

*s. seed bank = soil seed bank, longev. = plant longevity, veg. repr. = frequent vegetative reproduction, agam. = agamospermy

Supplementary table S1 (continued).

Species	Siring success[#]	Seed abortion	Low hybrid fitness	Overall clonality	Use of neopolyploids	Other reason for coexistence*	References
Chamerion angustifolium	y (h. 4x)	y	y	y	y (induced)		Husband and Schemske (1998, 2000); Burton & Husband (2000); Husband and Sabara 2004; Kennedy et al. (2006); Husband et al. (2008); Baldwin and Husband (2011, 2013); Sabara et al. (2013); Thompson et al. (2014, 2015)
Dactylis glomerata	-	(y)	-	(y)	y (natural)		Bretagnolle and Thompson (1996, 2001); Lumaret et al. (1987); Maceira et al. (1993); Bretagnolle and Lumaret (1995)
Dactylorhiza maculata s.l.	-	(n)	(n)	-	-		Ståhlberg (2009)
Deschampsia cespitosa	-	y	-	(y)	-		Rothera and Davy (1986)
Dianthus broteri	-	-	-	-	-		Balao et al. (2009)
Empetrum nigrum s.l.	-	-	-	-	-		Suda et al. (2004)
Galax urceolata	-	-	-	-	-		Burton and Husband (1999); Johnston et al. (2003)
Galium valdepilosum	-	-	-	-	-		Kolář et al. (2014)
Gymnadenia conopsea	y (h. 4x)	y	-	-	-		Jersáková et al. (2010); Trávníček et al. (2011, 2012); Gross and Schiestl (2015)
Heuchera cylindrica	-	-	-	-	-		Godsoe et al. (2013)
Heuchera grossulariifolia	-	(y)	-	-	y		Thompson et al. (1997); Segraves et al. (1999); Segraves and Thompson (1999); Nuismer and Cunningham (2005); Oswald and Nuismer (2010)
Hypochaeris incana	-	-	-	-	-		Tremesberger et al. (2009)
Ixeris nakazonei	-	-	-	-	-		Denda and Yokota (2004)
Jacobaea vulgaris	-	-	y	-	-		Hodálová et al. (2010, 2015); Mereďa et al. (2016)
Knautia arvensis	-	-	(y)	y	-	veg. repr.	Kolář et al. (2009, 2012); Hanzl et al. (2014)
Larrea tridentata	-	-	-	-	-		Laport et al. (2012, 2013, 2016); Laport and Ramsey (2015)
Lilium lancifolium	-	-	-	y	-		Kim et al. (2006); Chung et al. (2015)
Lippia alba	-	-	-	-	-		Reis et al. (2014)
Lotus alpinus	-	y	-	-	-		Gauthier et al. (1998)
Lythrum salicaria	-	-	-	-	-		Kubatova et al. (2008)
Melampodium cinereum	-	y	-	-	-		Stuessy et al. (2004)
Melampodium leucanthum	-	y	-	-	-		Stuessy et al. (2004)
Mercurialis annua	-	-	y	-	-		Buggs and Pannel (2006, 2007)

[#]h. 4x = higher for 4x cytotype

*veg. repr. = frequent vegetative reproduction

Supplementary table S1 (continued).

Species	Siring success[#]	Seed abortion	Low hybrid fitness	Overall clonality	Use of neopoly-ploids	Other reason for coexistence*	References
Odontites vernus	-	-	-	-	-		Koutecký et al. (2012)
Oxalis obtusa	-	-	-	y	-	veg. repr., longev.	Krejčíková et al. (2013)
Oxalis pes-caprae	-	y	-	y	-		Castro et al. (2007, 2013); Krejčíková et al. (2013); Ferrero et al. (2015)
Parasenecio auriculata	-	-	-	-	-		Nakagawa (2006)
Phleum pratense	-	-	-	-	-		Perný et al. (2008)
Pilosella echioides	y (h. 2x)	-	y	-	-	weak hyb. bar.	Peckert & Chrtek (2006); Trávníček et al. (2011); Chrtek et al. (2014); Herben et al. (2016)
Pilosellla rhodopea	-	-	-	-	-		Šingliarová et al. (2011)
Plantago media	-	(y)	-	-	y	longev.	Van Dijk 1992, Van Dijk & Van Delden 1990, Van Dijk & Bakx-Schotman 1997
Populus tremuloides	-	-	-	y	-		Mock et al. (2012)
Ranunculus adoneus	n	y	y	(n)	-		Baack (2004, 2005a, 2005b); Baack and Stanton (2005)
Ranunculus kuepferi	-	-	-	-	-		Cosendai and Horandl (2010); Cosendai et al. (2013); Kirchheimer et al. (2015)
Saxifraga oppositifolia	-	-	-	-	-		Muller et al. (2012); Eidesen et al. (2013)
Senecio carniolicus	-	-	-	-	-		Suda et al. (2007); Sonnleitner et al. (2010, 2013; 2016); Hülber et al. (2015)
Solidago altissima	-	(y)	-	(y)	-	disturb.	Halverson et al. (2008); Richardson & Hanks (2011); Ettreson et al. (2016)
Solidago gigantea	-	y	-	(y)	-	disturb.	Schlaepfer et al. (2008)
Spartina pectinata	-	-	-	(y)	-	disturb.	Kim et al. (2012, 2013)
Spergularia echinosperma	-	-	-	-	-		Kúr et al. (2012)
Symphytum tuberosum	-	-	-	-	-		Kobrlová et al. (2016)
Taraxacum sect *Taraxacum*	-	-	-	-	-		Meirmans (1999, 2003); Verdujin (2004); Martonfiova (2016)
Tripleurospermum inodorum	-	-	(n)	n	-	s. seed bank, immigr.	Kay (1969); Čertner et al. (2017)
Turnera sidoides pinnatifida	-	-	-	-	-		Elías et al. (2011)
Vaccinium oxycoccos	-	-	-	-	-	veg. repr., longev.	Suda (2003)
Vicia cracca	-	-	-	-	-		Trávníček et al. (2010); Eliášová and Munzbergová (2014); Eliášová et al. (2014)

[#] h. 2x = higher for 2x cytotype

*s. seed bank = soil seed bank, longev. = plant longevity, veg. repr. = frequent vegetative reproduction, agam. = agamospermy, disturb. = disturbance, immigr. = imigration of cytotypes (within a contact zone), weak hyb. bar. = weak interplody hybridization barriers