Matthias Hartmann

Geographical parthenogenesis: evolutionary and ecological significance of apomictic reproduction in plants

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Charles University • Faculty of Science, Department of Botany

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Geographical parthenogenesis: evolutionary and ecological significance of apomictic reproduction in vascular plants

Doctoral Thesis

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Supervisor: Patrik Mráz

Prague, 2018

> “GEOGRAPHICAL PARTHENOGENESIS IS OFTEN SEEN AS A SHORT TERM SUCCESS OF ASEXUALITY […] BUT THE EVOLUTIONARY RELEVANCE OF THE PHENOMENON IS STILL NOT WELL UNDERSTOOD.”
>
> E. Hörandl 2009 in ‘Lost Sex’

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DECLARATION

I declare that I have written this thesis independently using the mentioned references. It has not been submitted elsewhere, in full or in part, to obtain the same or other academic degree.

Matthias Hartmann

AUTHOR CONTRIBUTIONS

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Field work, assessment of seed herbivory, data analyses, writing original draft– 80%

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Field work, writing editing and review— 10%

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SUMMARY

Though associated with disadvantages such as limited adaptive potential in changing environments and accumulation of deleterious mutations, apomixis, i.e. asexual reproduction via seeds, bears the advantage of uniparental reproduction and the theoretical capability to found a new population with only one or a few individuals. Given the spatially and temporarily varying habitats plants face in the course of colonization and range expansion processes, it seems surprising that asexuals / apomicts are more frequently wider distributed compared to sexuals of the same or closely related species. Such geographical patterns are termed geographical parthenogenesis. Several hypotheses have been proposed trying to explain this phenomenon. However, geographical parthenogenesis remains still poorly understood, because investigations rather focused on single hypotheses, which were rather tested several times independently on multiple organisms than *vice versa*. Therefore, the present study aimed to tackle the phenomenon of geographical parthenogenesis from multiple angles, i.e. testing several hypotheses simultaneously using *Hieracium alpinum* as a model system. The occurrence of two cytotypes, diploid and triploid, within the same species, each of them tightly associated with a particular reproduction mode, i.e. sexuality and apomixis, respectively, the striking geographic parthenogenesis pattern and the autopolyploid origin of the asexual / apomicitc cytotype make *H. alpinum* an excellent model to study the evolutionary and ecological significance of apomictic reproduction in vascular plants. Importantly, unpublished results of phylogenetic / phylogeographic study have shown that triploids originated multiple times from diploid lineages which were genetically slightly different from the extant ones. Furthermore, asexual triploids likely evolved outside of the current range of extant diploids (Mráz et al. 2009). This implies that asexual triploids have had some fitness / colonization advantage(s), which allowed them to take over their ancestral diploid lineages, which had been in fact replaced by their triploid descedents reproducing asexually.

Therefore, the central question of this study is to determine what factor(s), intrinsic, i.e. those relevant to fitness / colonization ability, extrinsic (e.g. opportunity to colonize largely deglaciated habitats; different selective biotic / abiotic pressures) or combination of both, contributed to currently observed pattern of geographical parthenogenesis in this species.

In a large field survey, we visited more than 30 populations across large parts of the species' distributional range. Using field observations and measurements (e.g. selected plant traits and environmental variables), evaluations of collected material (e.g. potential and realized seed sets, ploidy level and the level of sexuality *versus* asexuality; size and velocity of seeds; carbon, nitrogen and their isotope composition in the leaves; phylogenetic relationship amongst individuals and populations) and experimental approaches (e.g. the germination experiment, growth and phenotypic plasticity experiment under controlled environmental conditions), the present thesis provides new insights into the phenomenon of geographical parthenogenesis.

More specifically, amongst the most important outcomes of the present thesis are (i) the evidence of obligatory apomictic reproduction in triploids and obligate sexual reproduction in diploids, and (ii) evidence for a higher and more stable reproductive assurance of asexual triploids. Our data thus suggest that uniparental reproduction advantage (so-called Baker's Law) together with the obligatory apomictic reproduction could, at least partly, explain the geographical parthenogenesis pattern of *H. alpinum*. Another important outcome of the present thesis are (iii) limited cytotype differences in many measured traits due to strong latitudinally and altitudinally driven effects of the local abiotic and biotic environments. In particular, the distinctiveness of Scandinavian when compared to Central European mountains in the abiotic and biotic environments was likely the cause for these observed patterns. For this reason, no support for the Red Queen hypothesis (i.e. between-cytotype differences in the level and amount of biotic interactions) and nearly no between-cytotype differences in seed and eco-physiological leaf traits were found in the framework of the present thesis.

This is fairly interesting, as polyploidization, accompanied by changes in the plants’ morphology, could alter the manner by which different cytotypes respond to the local abiotic and biotic conditions. Therefore, it remains unclear whether (i) the effect of ploidy, (ii) the effects of local environmental conditions or (iii) the combination of both could also have underlined the observed geographical parthenogenesis pattern in *H. alpinum*. Throughout the present thesis, I observed a “consistent inconsistency”, i.e. considerable intra- and inter-population variation. Such variation can most likely be explained by the large latitudinal and elevational gradient covered by our sampling across the species range because these gradients were accompanied by differences in abiotic and biotic conditions. Moreover, the pattern of “consistent inconsistency” implies considerable habitat heterogeneity within and between the sampled populations. Such habitat heterogeneity under natural conditions likely resulted in the observed considerable variation in the measured traits rather than clear-cut between-cytotype differences. Therefore, the resulted patterns could be due to plastic responses of plants to site-specific environment. Furthermore, the plastic response could be enhanced by adaptive processes. To give an example but one, the cytotypes followed opposing dispersal and germination strategies; whilst sexually derived seeds were potentially dispersed on shorter distances and did not exhibit dormancy, apomictically derived seeds exhibited the opposite characteristics. Interestingly, seed dormancy was most pronounced in Scandinavian populations, putatively indicating local adaptations.

As aforementioned, triploids likely replaced extinct diploids outside of the current range of diploids. Given the tight association between ploidy and reproduction mode in *H. alpinum*, it remains still unclear, how much of the putative enhanced fitness / colonization ability of triploids is due to (i) the higher and more consistent reproductive assurance, (ii) the polyploidization driven alteration in the plants' phenotype (alterations of morphology) and genotype, or (iii) the combination of both. The plasticity experiment should untangle this problem, as it should allow me to investigate whether sexual genotypes / clonal lineages react differently to the given environments or not. If the between-cytotype differences in plasticity are bigger than the differences between sexual genotypes / clonal lineages, then the fitness / colonization advantages due to polyploidization could play an important role in the geographical parthenogenesis pattern. In case of clear-cut differences between the sexual genotypes / clonal lineages, then sexual genotypes / clonal lineages adapted to the local environments they occur in. Moreover, this is of particular interest in the context of the ubiquitous clonal lineage, which has colonized Scandinavia and large parts of the Alps. I expect that this lineage will react the least in response to the different treatments in comparison to the sexual genotypes / clonal lineages, which would be in accordance with the general-purpose genotype hypothesis. Alternatively, a neutral (stochastic) scenario might have been involved, e.g. the ubiquitous clone was the most abundant clone in the course of the massive colonization process following glacial retreats.

Future work should focus on *H. alpinum*'s evolutionary history. More specifically, investigations with the focus on the genetic constitution of sexual genotypes / asexual clonal lineages as well as the intra-specific phylogenetic relationships (i.e. phylogeography and age of sexual genotypes / asexual clonal lineages) hold the potential to shed light on the mechanisms underlying the pattern of geographical parthenogenesis in this species and especially the success of the ubiquitous clonal lineage. Furthermore, given the important role of epigenetic processes involved in speciation / local adaptations through alternated gene expression, further research should be focused on epigenetic patterns in sexual genotypes and asexual clonal lineages. This would be especially important in the ubiquitous clonal lineage, which colonized a large part of the current distributional range.

INTRODUCTION

A rather finite number of animals and plants reproduce asexually, i.e. without syngamy of reduced gametes, which originated from meiosis, when compared to the number of sexually reproducing organisms. The resulting progeny of such processes is genetically uniform and represents a genetic clone of the mother organisms. Though the adaptation potential and lifespan should theoretically be limited, the asexual mode of reproduction also bares several advantages. Amongst others, given the male-independent reproduction, asexuals are able to found a new population with only a single or a small number of individuals. Consequently, this superior colonization ability of asexuals compared to sexuals, should be manifested in larger distributional ranges of the former – a pattern called geographical parthenogenesis. However, this distributional pattern remains poorly understood. Though several possible explanations for geographical parthenogenesis have been proposed and tested independently on several organisms of the animal and plant kingdoms the referring studies show a rather mixed support to these hypotheses, in dependence on the model system.

Therefore, the aim of the present thesis is to contribute to unraveling the phenomenon of geographical parthenogenesis in plants. Importantly, within the frame of the present thesis, asexuality is understood solely as apomixis, i.e. asexual reproduction via seeds. The thesis is arranged into two main parts: In the introductory chapters, I will give a short overview about the reproduction in plants and highlight the differences between sexual and asexual / apomictic reproduction and their consequences. Subsequently, I will focus on apomixis, its types, and their evolutionary consequences, as well as hypotheses about the origin of apomixis. Then I will briefly summarize the hypotheses underlying geographical parthenogenesis, and introduce my model system on which I have tested several of the previously introduced hypotheses. I will outline the aims and questions of my thesis and summarize the methodologies and outcomes of my approaches. The second part of the thesis encompasses four papers, each of them represent either already published or unpublished (resubmitted) manuscripts. At the end of the thesis, I have included as an Appendix, my brief CV with a list of my publications.

PRINCIPLES OF REPRODUCTION IN PLANTS

Sex is the predominant reproduction mode of eukaryotic life forms, in which two genetically distinct reduced female and male gametes fuse to form a genetically distinct progeny. This process is called mixis or syngamy (Bell 1982; Colegrave 2012). Sex putatively enhances the evolutionary potential of a species as it creates novel gene combinations, which consequently may become beneficial in a temporarily and spatially changing environment. On an individual level, it holds the potential to eliminate deleterious mutations (Crow 1994). On the other hand, "[…] sex is actually a costly process that ought to be lost quickly from populations" (Colegrave 2012). This phenomenon is frequently referred to as the 'two-fold cost of sex' (Maynard Smith 1978). Recombination putatively breaks up beneficial co-adapted gene combinations making the progeny less well-adapted to the environment when compared to the parents (the cost of meiosis or recombinational load; Colegrave 2012). Secondly, from the individual point of view, sex requires the production of males which only serve as gamete donor but do not give birth to progeny and need to be met by females (the cost of males; Lively 2010). Moreover, costly specialized reproductive structures and mechanisms need to be produced (Williams 1971). Asexual reproduction bears the disadvantage of accumulation of deleterious mutations, so-called Müller's ratchet (Müller 1964). As asexuals lack recombination, their whole genome acts as a giant linkage group, i.e. selection acts upon the whole genome / the individual rather than on certain genes. Furthermore, because of reduced genetic / genotypic diversity on which selection may act upon, asexuals' have a reduced adaptive potential in changing environments (e.g. Hörandl 2006).

SEXUAL REPRODUCTION

The principle of gamete production applies to female and male gametes. In a diploid individual, diploid precursor cells undergo meiosis resulting in haploid cells, which further undergo mitosis. In plants, the male gametes differentiate from many precursor cells in the anthers (pollen mother cell, PMC). By contrast, usually only one female precursor cell (megaspore mother cell, MMC) differentiates into the female gametophyte (i.e. embryo sac) in the ovule. The seven-nucleate *Polygonum*-type represents the most common form of embryo sacs, occurring in more than 70% of angiosperms (Hand and Koltunow 2014). In a diploid plant, the meiosis of the diploid MMC yields, through a process called megasporogenesis, in four haploid megaspores (tetrads), out of which three degenerate and one undergoes three mitotically divisions without cytokinesis in the process called megagemetogenesis. Subsequently, the eight haploid nuclei migrate and cellularize, forming three antipodes with unknown function at the chalazal pole and two synergids and one egg cell at the micropyle pole. The synergids attract the male gametes and the egg cell fuses with the male gamete to form the diploid embryo. The remaining two nuclei fuse to a large central or polar nuclei, forming a homo-diploid precursor nucleus of the endosperm, and subsequently fuse with a second male gamete to form the triploid endosperm. This double fertilization is necessary to start endosperm development and embryogenesis, respectively (Berger et. al 2008). The resulting endosperm exhibits a maternal paternal ratio of 2:1, which is needed for successful endosperm formation. After successful fertilization, a seed is formed which comprises the seed coat, diploid embryo, and triploid endosperm.

ASEXUAL SEED PRODUCTION — APOMIXIS

Interestingly, certain plant genera are able to produce seeds asexually, i.e. by omitting meiosis and syngamy. The asexual reproduction of plants via seeds is called apomixis (Winkler 1908) or agamospermy (Täckholm 1922). Already over a century ago, in 1841, the observations in female individual of *Alchornea ilicifolia* (syn. *Caelebogyne ilicifolia*; Euphorbiaceae) revealed that this individual was able to produce seeds independently of males (Smith 1841; Asker and Jerling 1992). Winkler (1908) provided the first rather broad definition of apomixis. The modern definition of apomixis is the production of seeds which are formed from unreduced female gametes parthenogenetically, i.e. without fertilization (Nogler 1984; Asker and Jerling 1992). Each offspring is thus genetically identical to the mother plant. Apomixis evolved repeatedly and independently in multiple genera (Carman 1997). Around 2.2% or 293 of angiosperm genera contain apomictic species out of which most belong to the three families: Asteraceae, Rosaceae and Poaceae (Hojsgaard et al. 2014).

TYPES OF APOMIXIS

Two main mechanisms of apomixis can be distinguished (sporophytic and gametophytic apomixis, respectively), sharing the following three key features: (i) generation of a cell capable of forming an embryo without meiosis / bypassing meiosis during embryo sac formation (apomeiosis), (ii) spontaneous development of an embryo independent of fertilization (parthenogenesis), and (iii) independent or fertilization-dependent formation of endosperm (Koltunow and Grossniklaus 2003).

In the case of sporophytic apomixis, also often referred to as adventitious embryony, the embryo emerges parthenogenetically from the ovule surrounding sporophytic tissue (Naumova 1992). After the stimulation of the sexual embryo and endosperm development, the growth of multiple apomictic pro-embryos or embryo initial cells is induced, which, subsequently, invade the embryo sac and eventually outcompete the sexually derived embryo “hijacking” the sexually derived endosperm (Richards 1997). It is important to note that sexually and apomictically derived embryos can co-occur within the same ovule (polyembryony). This type of apomixis is still not fully understood and is common mainly in subtropical / tropical shrubs and trees with fleshy fruits, e.g. mango or citrus (Hand and Koltunow 2014).

In gametophytic apomixis, the embryo derives fertilization-independently, from an unreduced egg cell within the embryo sac. The formation of the endosperm may be fertilization-dependent or independent. Based upon on the tissue from which the embryo sac differentiates, two types of gametophytic apomixis can be distinguished, namely apospory and diplospory. The aposporous embryo sacs originate from one or multiple cells (aposporous initial; AI) in the nucellus in close proximity to the sexual embryo sac (Koltunow 1993). After mitosis, the AI forms an embryo sac of the *Polygonum*-type and the sexually derived embryo sac degenerates (Hand and Koltunow 2014). Importantly, sexual and aposporous processes coexist within the same ovule (Koltunow 1993). This type of apomixis occurs in e.g. *Poa* (e.g. Åkerberg 1939), *Potentilla* (Müntzing and Müntzing 1941), *Rubus* (Nybom 1988), *Sorbus* (Jankun and Kovanda 1986; 1987; 1988), *Cotoneaster* (Sax 1954) or *Pilosella* (Rosenberg 1908). In contrast to apospory, diplospory is the considerably less frequent form of gametophytic apomixis Hojsgaard et al. 2014). In diplosporous plants, the embryo originates from archesporial cells and the surrounding endosperm is formed autonomously. Meiosis is either initiated but is restitutional at the end of meiosis I (*Taraxacum*-type), like e.g. in the genera *Taraxacum* and *Chondrilla* (van Dijk 2003), or the archesporium cell undergoes mitosis directly to form the embryo sac (*Antennaria*-type) (Richards 1997), such as in *Antennaria* (Asker 1980; Bayer and Stebbins 1983; Bayer 1987), *Erigeron* (Gustafsson 1946; Gustafsson 1947; Harling 1950) or *Hieracium* s.str. (Skawińska 1963; Hand et al. 2014). In his review about naturally occurring apomixis in Asteraceae, Noyes (2007) provided evidence that diplospory is undoubtedly the predominant type of apomixis in this family as it occurs in 18 out of the 22 genera exhibiting apomixis. Though no phylogenetic clustering of this trait was observed, these genera are mostly confined to the tribe of Asteroideae, with the exception of the tribe Lactuaceae. Apomixis occurs throughout the family and is restricted to polyploids (Noyes 2007).

Importantly, diplosporous and aposporous taxa differ in their level of strictness of apomixis. The former type of apomixis is more obligatory than the latter due to the co-occurrence of reduced (meiotically) and unreduced (mitotically) MMC within the same ovule in aposporous apomicts (Noyes 2007). The residual sexuality of aposporous apomicts can have important evolutionary and ecological consequences as it holds the potential to increase the apomicts' genotypic and phenotypic variability. In the genus *Pilosella* (former *Hieracium* subgen. *Pilosella*), Krahulcová et al. (2000) and Bicknell et al. (2003) showed that facultative apomixis can lead to the formation of several different types of progeny. Hybrids may arise when an unreduced egg is fertilized, or a reduced egg undergoes parthenogenesis, putatively altering the ploidy of progeny compared to the mother plant (so-called haploid parthenogenesis). Accordingly, Houliston and Chapman (2004) reported that 58% of the genetic diversity of facultative *Pilosella* plants is explained by the level of residual sexual reproduction.

ORIGIN OF APOMIXIS

Given the great ecological and evolutionary potential of apomixis (e.g. fixation of hybridogenous progeny), the molecular mechanisms underlying apomixis are still not fully understood (e.g. Grossniklaus et al. 2001; Koltunow 1993). Over 70 years ago, Stebbins (1941) proposed necrohormones, genetic factors, hybridization and polyploidy as putative explanations for the molecular mechanisms underlying apomixis. More recent studies suggest that apomixis is heritable and under genetic or epigenetic control (van Dijk et al. 1999; Noyes and Rieseberg 2000; Albertini et al. 2001; Grimanelli et al. 2001; Koltunow and Grossniklaus 2003; Curtis and Grossniklaus 2007; Schallau et al. 2010; Conner et al. 2013). Koltunow and Grossniklaus (2003) reviewed several hypotheses about the origin of apomixis. Out of these, the most prominent hypotheses are those proposed by Carman (1997, 2001) and Nogler (1984). The "genome-collision-hypothesis" states that hybridization between two (or more) previously isolated ecotypes or species, differing in timing of flowering and developmental pathways, may lead to an asynchronous expression of genes regulating the florogenesis and therefore may cause apomixis (Carman 1997; Carman 2001; Grimanelli et al. 2001). Nogler (1984) postulated that apomixis is controlled by a single dominant Mendelian factor, lethal in a haploid state, preventing haploid gametes to transfer apomixis to the next generation, which has received considerable support given the strong association between apomixis and polyploidy (Asker and Jerling 1992).

GEOGRAPHICAL PARTHENOGENESIS

Interestingly, the shift from sexual to asexual reproduction via seeds has frequently resulted in a shift in the distributional success of some groups with apomicts being wider distributed when compared to sexuals, contesting the general idea of asexuality as an "evolutionary dead end" (Maynard Smith 1978). Nearly a century ago, based upon parthenogenetic arthropods, Vandel (1928) described this phenomenon as geographical parthenogenesis ("parthénogenèse géographique"). Ever since geographical parthenogenesis describes the geographical separation (or trends) between sexually and asexually reproducing organisms within one species (or between closely related taxa), whereby asexual organisms, compared to sexual, have larger distributional ranges. Asexuals occur rather at higher latitudes and altitudes (Ghiselin 1974) and are more frequently in marginal and extreme habitats (previously glaciated areas, desert) than sexuals (Cuellar 1977; Lynch 1984; Bierzychudek 1985; Peck et al. 1998; Parker 2002; Kearney 2005; Hörandl 2006).

Geographical parthenogenesis is exhibited by animals such as the small Crustacea of the *Daphnia* genus (e.g. *Daphnia pulex*; Beaton and Paul 1988), the grasshopper *Warramaba virgo* or the gecko *Heteronotia binoei* (Vorburger 2006). For further examples of animals see e.g. Kearney (2005). An early large scale comparison of distributional ranges between sexual and closely related asexual plant species was conducted by Bierzychudek (1985). In the subsequent decades, geographic parthenogenesis has been confirmed for several plant taxa, e.g. *Ranunculus kuepferi* (Cosendai and Hörandl 2010), the *Ranunculus auricomus* complex (Hörandl and Paun 2007), *Townsendia hookeri* (Thompson and Whitton 2006), *Antennaria* (Bayer 1991), and *Hieracium alpinum* (Mráz et al. 2009).

HYPOTHESES UNDERLYING GEOGRAPOHICAL PARTHENOGENESIS

Importantly, as noted by Hörandl (2006) "The phenomenon […] has long been recognized, but it is still not well understood." Consequently, several non-exclusive hypotheses have been proposed trying to explain these patterns, which can be assigned to the following three groups:

(i) The first group refers to advantages of asexuals on the genetic level. In highly fragmented or subdivided marginal habitats, sexuals, but only sexuals, will suffer from inbreeding depression, due to the limited number of putative mating partners. In populations of small size or during colonization events, mating between closely related individuals leads to genome-wide increasing levels of homozygosity and therefore a decrease in an individual's fitness. This is partly because deleterious alleles will accumulate and are more probable to become homozygous after inbreeding (Charlesworth and Willis 2009). On the contrary, asexuals will be protected from such founder events and therefore, they will be better colonizers than sexuals. This assumption is called the **metapopulation hypothesis** (Haag and Ebert 2004). Moreover, assuming habitat differences between central and marginal populations, sexual marginal populations are exposed to so-called outbreeding depression as a consequence of gene flow from central populations. Such gene flow putatively weakens the level of adaptation in sexual marginal populations. Contrarily, asexual marginal populations are not exposed to the danger of such swamps of gene flow (Wallace 1959). However, the metapopulation hypothesis has only received little attention. To the best of my knowledge only the studies by van der Merwe et al. (2010) on the heterostylous *Erythroxylum pusillum* and by Dering et al. (2017) on the grey alder *Alnus incana* (L.), provide some evidence for the metapopulation hypothesis.

Given the strong association between polyploidy and apomixis (Asker and Jerling 1992), Bierzychudek (1985) proposed that the distributional success can best be explained by whole genome duplication accompanied by a higher level of genetic variation (heterozygosity assurance; Haag and Ebert 2004; Vrijenhoek 1985) and therefore increased ecological tolerance. Furthermore, polyploidization can putatively alter a species' micro and macro phenotype (i.e. different sizes of cell compartments and plant body) and biochemistry (Levin 2002; Comai 2005; Parisod et al. 2010) affecting polyploids' e.g. dispersal (Linder and Barker 2014) and competitive abilities (Maceira et al. 1993; Collins et al. 2011; Eliášová and Münzbergová 2017) or germination behavior (Bretagnolle et al. 1995; Hoya et al. 2007; Haouala et al. 2009; Eliášová and Münzbergová 2014). As polyploidy is not correlated with larger distribution areas in sexual polyploid plants, Bierzychudek's (1985) hypothesis has been challenged (Stebbins and Dawe 1987; Hörandl 2006; Martin and Husband 2009).

(ii) Two mutually non-exclusive hypotheses regarding the exploitation of ecological niches have been proposed (Vrijenhoek and Parker 2009). The common basis of both hypotheses is how the selection acts upon individuals. In sexual populations, selection acts upon individual genes with small additive effects, which, consequently, change in frequency. In contrast, in asexual populations, selection acts upon the individuals' whole genome leading to an exclusion of asexual genotypes from the population (Hörandl 2006). Sexuals encompass a short-term advantage since those populations remain genetically variable and flexible through continuous recombination and therefore reshuffling of genetic combinations, optimizing the partitioning of resources amongst individuals in a spatially heterogeneous habitat. In other words, the niches of individuals in a sexual population only overlap to a small extent. In the events of mutations in meiosis controlling genes or hybridization, such a population can give rise to asexual clones. The derived clones freeze a certain portion of the sexual ancestors' niche and therefore partition the available resources amongst each other, whereby each asexually derived clone will have a narrower ecological niche compared to the sexual ancestors (Butlin 2002). Further inter-clonal selection favors clones in a way to minimize the niche overlap, i.e. several specialized clonal lineages, fixing an array of clones and leading to a stable coexistence between sexual and asexual plants. This selection process is frequency and density dependent (i.e. soft selection). Given the advantage of reproductive assurance of asexuals, a frequent formation of clones can ultimately lead to the exclusion of sexual plants from the population, so that an asexual population consisting of several specialist clonal lineages will persist (**Frozen niche-variation hypothesis**; Vrijenhoek 1979; Vrijenhoek 1984). By contrast, in temporarily variable environments, selection processes are frequency and density independent (i.e. hard selection). Given the selection on the whole genome level in asexuals, the **general-purpose genotype** (Parker Jr et al.1977) or **jack-of-all-trades hypothesis** (Oplaat and Verhoeven 2015) proposes that selection will favor clones with the broadest ecological niche, i.e. those clonal lineages which are generalists. Importantly, such clones do not necessarily exhibit the highest fitness across all environments but rather survive in all environments. It is thought that a generalist clone is favored in disturbed habitats or in situations of low competition with its sexual progenitors (Vrijenhoek and Parker 2009). The general-purpose genotype hypothesis has frequently been tested in animal taxa (for a list of studies see Vrijenhoek and Parker 2009). To my knowledge, only four studies have been conducted in the plant kingdom so far, which are either in favor of the hypothesis (Bierzychudek 1989; Oplaat and Verhoeven 2015) or not (Kenny 1996) or show mixed support (Michaels and Bazzaz 1989). Concerning the frozen niche-variation hypothesis, the selection of asexually derived clones in a non-overlapping of ecological niches manner is harder to test. Studies on only two plant taxa have been conducted, namely *Taraxacum officinale* (Solbrig 1971; Solbrig and Simpson 1974; Vavrek et al. 1996) and *Erigeron annuus* (Stratton 1994). The results of both studies were in favor of this hypothesis.

(iii) The last group of hypotheses refers to advantages in colonization abilities and biotic interaction. **Baker's Law** (Baker 1955) postulates superior reproductive assurance and colonization abilities of uniparentally reproducing organisms. As mentioned above, asexual organisms have a two-fold-advantage over sexual reproducing organisms (Maynard Smith 1978). Given the partial pollen-independency of aposporous apomicts (pseudogamy) or complete pollen-independency of diplosporous apomicts (Asker and Jerling 1992) in a heterogeneous pollinator environment (i.e. competition for pollinators or a lack of pollinators under unfavorable weather conditions), theoretically, one population can be found by only a single uniparentally reproducing individual (Baker 1955). Surprisingly, Baker's Law has only been rarely assessed in sexual-asexual plant complexes. Published studies comparing the seed set of sexual *versus* apomictic plants provide rather mixed support for increased reproductive assurance in asexuals. Whilst no difference in the seed set between sexual and apomictic *Antennaria parlinii* were found in natural populations, in a pollinator free greenhouse, sexual only set seeds after manual pollination (O'Connell and Eckert 1999). In natural populations of *Taraxacum* sect. *Ruderalia*, apomicts consistently exhibited higher seed sets over a three year period (van Dijk 2007).

In the co-evolutionary race of arms between pathogens and hosts and between competitors (Ebert and Hamilton 1996), sexually reproducing organisms are supposed to have an advantage over asexually reproducing organisms due to an increased adaptive potential, and continuous evolution through recombination (Butlin 2002). This is of particular importance in habitats with many biological interactions. Asexuals should have an advantage at species' margins and more extreme habitats with less biotic interactions (Asker and Jerling 1992). Moreover, the biotic pressures (e.g. competitors, parasites and pests, but also pollinator activities) are supposed to decline with increasing latitudes and altitudes and therefore asexual organisms should be favored in those regions (e.g. Glesener and

Tilman 1978; Bell 1982; Asker and Jerling 1992). Van Valen (1973) coined this relationship in the **Red Queen hypothesis**. This hypothesis received only a little attention in the plant kingdom (but see Verhoeven and Biere 2013; Maron et al. 2018).

THE GENUS *HIERACIUM*

Formerly, the genus *Hieracium* L. (hawkweed) consisted of *Hieracium* subgen. *Chionoracium* Dummort., *Hieracium* subgen. *Hieracium* L. and *Hieracium* subgen. *Pilosella* (Hill) S. F. Grey (e.g. Zahn 1921-1923, Sell and West 1976). However, the latter of which, based on morphological and genetic distinctiveness, is now treated as an independent genus (Bräutigam and Greuter 2007). The focus of the present thesis is on *Hieracium* s. str., i.e. excluding the subgenera *Chionoracium* and *Pilosella*, which belongs to one of the most complex genera of vascular plants (Royal Botanical Gardens 1993; Chrtek et al. 2004).

The first observations of apomixis in this genus can be traced back to J. G. Mendel. He was the first to observe apomixis in *Hieracium* s.l., though he was not aware that the patterns he obtained in experimental crosses were the results of apomictic reproduction. Whilst artificial hybridizations in the closely related genus *Pilosella* produced true hybrids, i.e. with morphological characters of both parental species and mother-like progeny, crosses between *H. umbellatum* and *H. vulgatum* as well as between *H. murorum* and *H. umbellatum* produced progeny confined to either parental species’ morphology. Mendel concluded that selfing was the cause for this outcome, although it was likely agamospermy (Mendel 1950). Using castration experiments (emasculation by cutting the upper half of the capitulum with a razor blade before flower opening), Ostenfeld and Raunkiær (1903) and Murbeck (1904) provided the first empirical evidence of apomixis in this genus. The occurrence of diplospory of the *Antennaria*-type in *Hieracium* was cytoembryologically proven for the first time by Bergman (1935) and later by

Skawińska (1963). Given the frequent polyploidization and hybridization in this genus, leading to reticulate patterns of morphology and genetic diversity (Mráz 2001; Chrtek, et al. 2004; Fehrer, et al. 2005; Noyes 2007; Fehrer et al. 2009; Krak et al. 2013), the taxonomy of this genus went through a lot of changes. Moreover, the occurrence of apomixis throughout the genus adds a further factor to the taxonomic turmoil because "apomictic units do not fit the biological species criterion" (Schuhwerk 2002). Depending on the taxonomist school (excluding *Pilosella*), between 500 and 5000 species have been considered (Majeský et al. 2017). The British and Scandinavian taxonomy is rather complex with thousands of microspecies reported from Scandinavia only (Sell and West 1976; Tyler 2001; Tyler 2006). In contrast, the so-called Central European taxonomic school is based on Zahn's wider taxonomy concept by dividing *Hieracium* taxa into two groups of species (Zahn 1921-1923).

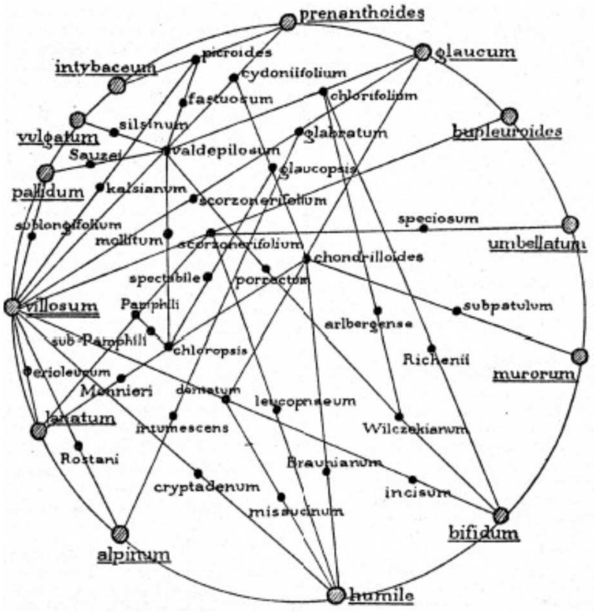

Figure 1: Diagram with selected *Hieracium* species, divided into "Hauptarten" (basic species; big dashed dots, underscored) and "Zwischenarten" (intermediate species; small black dots). The diagram shows the putative origin (hybridogenous) of intermediate species. Figure taken from Zahn (1921-1930).

"Hauptarten" or basic species (species principales) are characterized by a unique morphology and are supposed to serve as parents for "Zwischenarten" or intermediate species (species intermediae) which display a combination of morphological characters of two or more “Hauptarten”. A schematic overview is of Zahn’s taxonomy is given in Figure 1. The base chromosome number in *Hieracium* s.str. is x = 9 (Gustafsson 1946; Gustafsson 1947; Merxmüller 1975; Asker and Jerling 1992). The prevailing cytotypes are triploid (2n = 3x = 27) and tetraploid (2n = 4x = 36), whilst diploids (2n = 2x = 18) are relatively rare and pentaploids have been recorded but are extremely rarely (Mráz 2001; Chrtek et al. 2004). Importantly, diploids are confined to rather geographical restricted areas but triploids occur throughout the European distributional range (Merxmüller 1975).

HIERACIUM ALPINUM AS A MODEL SPECIES FOR STUDYING GEOGRAPHICAL PARTHENOGENESIS

In the present thesis, which is focused on testing mechanisms underlying the geographical parthenogenesis in plants, *Hieracium alpinum* from *Hieracium* sect. *Alpina* has been used as a model species (Figure 2).

Figure 2: *Hieracium alpinum* (Munții Făgărașului, 2014). Photo credit: P. Mráz.

Hieracium alpinum (alpine hawkweed; Figure 2) is an arcto-alpine perennial herb of the alpine or rarely subalpine belt growing on acidic soils in alpine grasslands, dwarf shrub communities, rock ledges and bare stone slopes (Figure 3).

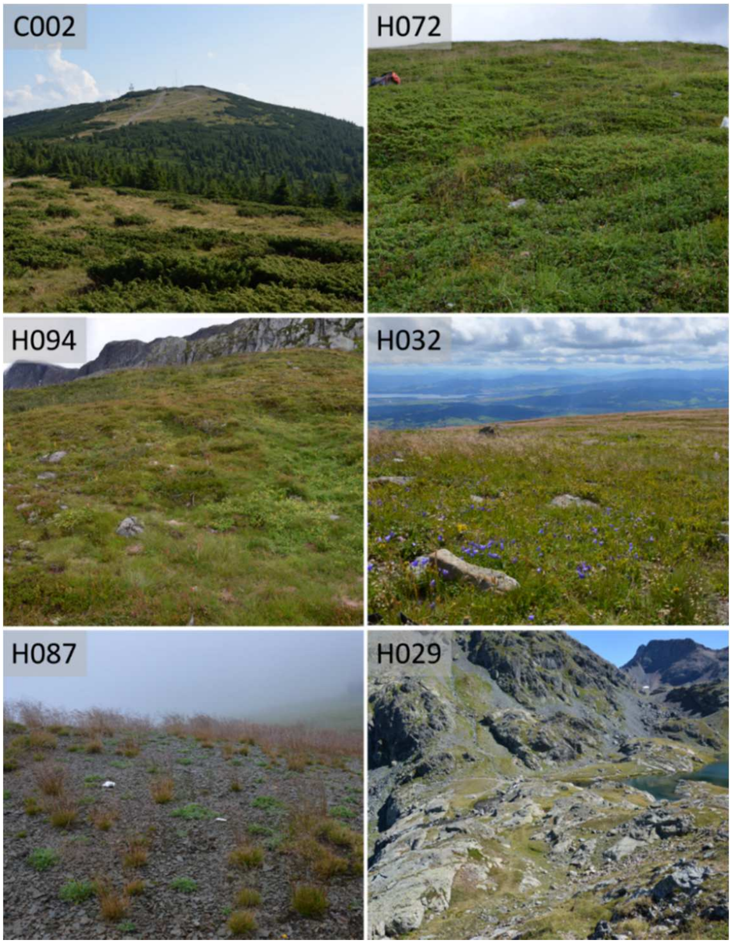

Figure 3: Habitats of *Hieracium alpinum* in its diploid range: **C002** (Mt. Vlădeasa, Romania; taken in 2014); H072 (Munţi Făgărașului, Romania; taken in 2014) and its triploid range: **H094** (Telemark, Norway; taken in 2014); **H032** (Mt. Babia hora, Slovakia; taken in 2015); **H087** (Vranica planina, Bosnia and Herzegovina taken in 2014); **H029** (Les lacs du Doménon, France; taken in 2015). Photo credits: P. Mráz.

Its distributional range extends from Northwestern Russia, through the Scandinavian and British mountains to Iceland and Greenland. *Hieracium alpinum* occurs also throughout the Central European mountains (the Alps, the Carpathians, the Sudetes) with some isolated populations in the Vosges, the Harz mountains and the Vranica Plateau in Bosnia and Herzegovina (Bräutigam and Schuhwerk 1992; Chrtek 1997; Chrtek and Plačková 2005). It forms rosettes with numerous leaves covered by scarce to dense simple hairs above. One rosette usually bares one leafless erect or ± curved stem, 6-24 (-30) cm high, with one ± globular entomophilic capitulum with yellow florets, enclosed by broadly linear and obtuse involucral bracts. In average, 60 pappus containing anemochorous achenes (i.e. one-seeded fruits) per capitulum are produced in the field (Mráz et al. unpubl.). Ripe achenes are dark brown to black and around 3.5-4mm long. Vegetative reproduction via accessory rosettes can also occur (pers. observation, Figure 4). The plants are pollinated by generalist pollinators belonging mostly to Diptera and Hymenoptera, rarely Lepidoptera orders (Mráz P., pers. observations).

Figure 4: Young accessory rosettes (red circles) in triploid *Hieracium alpinum* from the Vranica Planina, Bosnia and Herzegovina (Photo credit: P. Mráz).

The species encompasses two cytotypes: diploids and triploids, an extraordinary feature, as other species of *Hieracium* s.str. are usually confined to one cytotype only (Mráz at al.2009). As typical for this genus, the ploidy level is highly associated with the reproduction mode (Mráz and Zdvořak 2018). In *H. alpinum*, diploids reproduce obligatory sexually (Chrtek 1997; Mráz 2003), i.e. they exhibit regular micro- and megasporogenesis and are characterized by a sporophytic incompatibility system (Schuhwerk 1996; Mráz 2003). Triploids reproduce almost obligatory asexually exhibiting diplosporous apomixis of the *Antennaria*-type (Bergman 1941; Skawińska 1963). The inheritance of apomixis in *Hieracium* s.str is still unknown (Hand and Koltunow 2014). Importantly, the microsporogenesis in triploid plants is highly disturbed resulting in pollen of different size and quality (Chrtek 1997; Mráz et al. 2005; Mráz et al. 2009). As reported also for other *Hieracium* species (Pugsley 1948), reasons for this abnormality in microsporogenesis are the retardation of meiosis, precocity of meiotic divisions and, in case of odd ploidies, failure of chromosome pairing and contraction due to an odd number of chromosomes (Aparicio 1994). In contrast to unpollinated sexual Hieracia (Hand and Koltunow 2014), apomictic triploids are capable of autonomous endosperm formation, i.e. are pollination independent (Nogler 1984). Triploid apomicts omit the female meiosis and are, as reported for other Hieracia (e.g. Hand et al. 2014), characterized by precioucous embryony, i.e. the formation of the embryo starts before the flower opens (Skawińska 1963). However, the faculty of the apomictic reproduction remains unresolved; Skawińska (1963) reported high levels of seed abortion in triploids of *H. alpinum* occurring in the Tatra mountains, whilst emasculation experiments conducted by Chrtek (1997) yielded in rather high seed set in triploids. Given the differences in breeding systems, accompanied by limited gene flow due to the poor pollen quality, apomicts are not confined to serve as a putative parent in hybridization events nowadays (Mráz et al. 2005; Mráz and Paule 2006).

Despite several attempts (Shi et al. 1996; Stace et al.1997; Štorchová et al. 2002; Chrtek and Plačková 2005), the genetic differentiation and variation across the whole distributional range of *H. alpinum*, i.e. diploids as well as triploids, remain largely unknown. Studies conducted within the triploid range reported a decline in genetic diversity from the East to the West (Štorchová et al. 2002) and considerable intra- and inter-population genetic variation (Chrtek and Plačková 2005), thus suggesting the existence of multiple apomictic clones in *H. alpinum*. Preliminary genetic studies based on nuclear internal transcribed spacers (ITS) and AFLP markers have suggested that triploids are of autopolyploid origin and likely represent relicts of ancient extinct diploid lineages, which had occurred outside of the current diploid range and were replaced by the newly arisen triploids, suggesting superior fitness and / or colonization of triploids (Mráz et al. unpubl.). Furthermore, the molecular data indicated the occurrence of a widespread ubiquitous apomictic clonal lineage, which represented over 95% of the sampled plants in Scandinavia, Scotland, Greenland, and Iceland and by over 40% of the plants sampled in the Alps. A few plants of this clone have also been found in the Western Carpathians (Mráz et al unpubl.).

Importantly, though the habitats of both cytotypes are fairly similar, their distribution is allopatric (Mráz et al. 2009). Diploids are confined to the area of the Eastern and Southern Carpathians (Romania and Ukraine), whilst the triploids occupy the rest, and much larger part of the species distributional range (Figure 5), making “*Hieracium alpinum* a clear-cut example of geographical parthenogenesis” (Mráz et al. 2009).

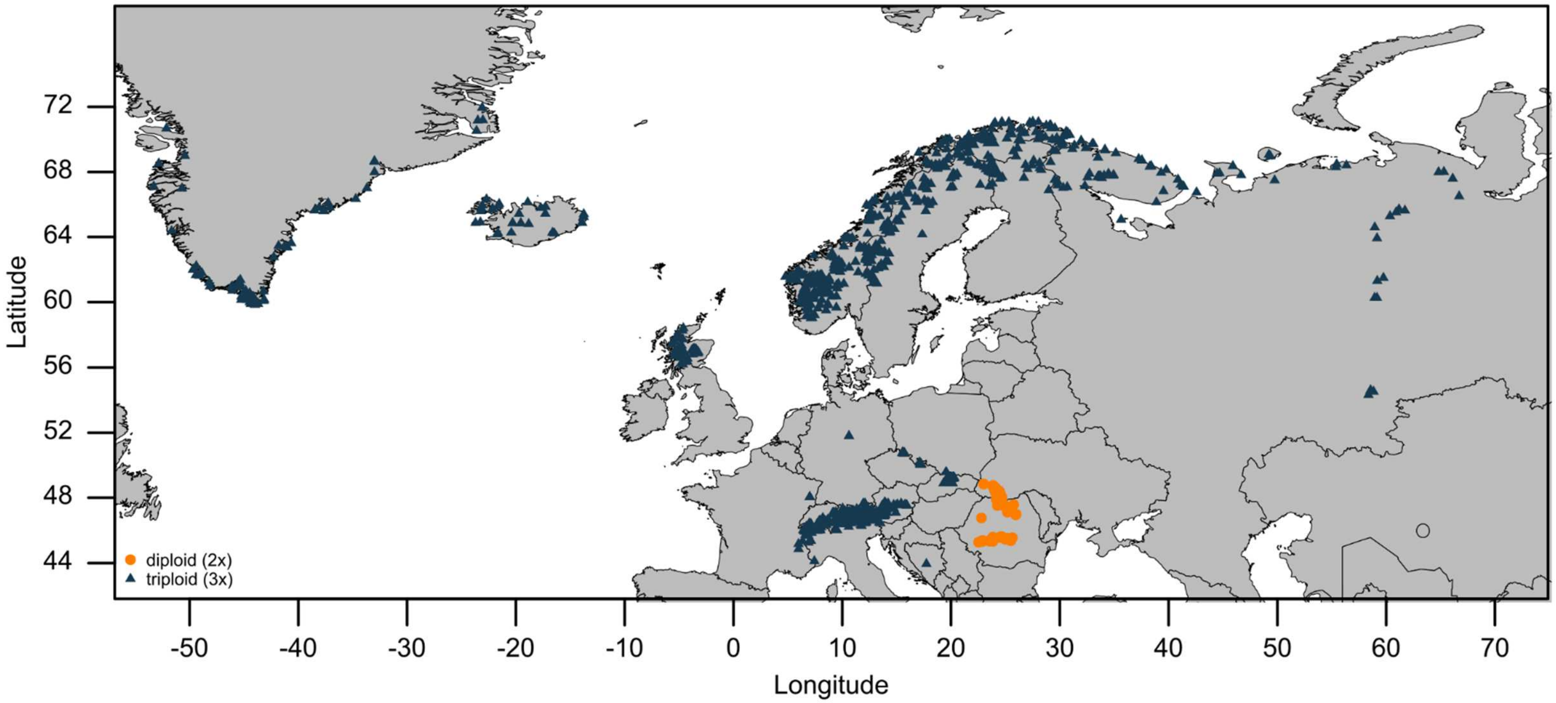

Figure 5: Distribution map of *Hieracium alpinum*'s cytotypes based on herbarium revisions done by J. Chrtek and P. Mráz (unpubl.) which includes 1244 specimens housed in 29 herbaria and the map published by Samuelsson (1954).

OBJECTIVES OF THE THESIS

Though several hypotheses trying to explain geographical parthenogenesis have been proposed, the mechanisms underlying geographical parthenogenesis remain insufficiently understood (Hörandl 2006). Therefore, this thesis aims to contribute to the unraveling this phenomenon using *H. alpinum* as a model system. *Hieracium alpinum* represents an excellent model organism to study this phenomenon, i.e. to evaluate the evolutionary and ecological significance of apomictic reproduction in vascular plants for the following reasons: (i) This species encompasses two ploidy levels, which are closely associated with the reproduction mode, i.e. sexual diploids *versus* apomictic autotriploids; though it is unknown to which extent triploids are apomictic. (ii) The cytotypes differ in their distribution with triploids being much wider distributed than diploids (Figure 5). This allopatric distribution raised several questions: Do the cytotypes' habitats differ in biotic and abiotic characteristics? Do the cytotypes differ in the response to local and controlled environmental conditions? Do the cytotypes differ in their reproductive assurance and dispersal potential? Finally, it raised questions about the origin of the triploid cytotype and the evolutionary history of the species, which could help us in understanding the allopatric distribution of cytotypes.

More specifically, the aim of my thesis is to answer the following questions:

(1) Are apomictic plants exposed to a lower biotic pressure than sexual plants? Does the level of biotic pressure follow a latitudinal and/or altitudinal clines?

(2) Do allopatric diploids and triploids differ in their eco-physiological responses to the local environmental conditions?

(3) Does the response to the local environmental conditions vary in a greater extent in triploids than in diploids?

(4) Do apomictic plants have a higher and more stable realized seed set compared to sexual plants due to their independence on pollinators?

(5) Do apomictic plants have a better dispersal ability than sexual plants?

(6) Does the level of phenotypic and phenological plasticity differ among apomictic clones and between apomictic clones and sexual genotypes when exposed to different controlled environmental conditions?

(7) Do apomictic plants show general purpose-genotype or frozen niche-variation behavior in reaction to different controlled environmental conditions?

(8) What is the evolutionary history / phylogeography of *Hieracium alpinum*?

BRIEF SUMMARY OF MAIN OUTCOMES OF THE THESIS

To answer these questions, we visited more than 30 populations from Nordcap, Norway in the North to the Vranica Plateua, Bosnia and Herzegovina in the South and from the French Alps in the West to the Eastern Carpathians in Romania in the East, covering large parts of the distributional range of *H. alpinum.* In each population, we collected capitula with ripe achenes and leaf samples and measured several individual traits and habitat characteristics. I used the gathered data to answer the aforementioned questions. Out of the outcomes, I will only shortly outline published / submitted manuscripts with details provided in those publications (questions 1 to 5). As for still unpublished studies (questions 6 to 8), I will provide methodological details and an overview of the progress of the analyses.

Question 1

The Red Queen hypothesis suggests that sexual organisms, when compared to closely related asexual organisms, occur in habitats with higher biotic pressures due to the evolutionary advantage of the former. The biotic pressure is supposed to decline with increasing latitude and altitude. I tested the Red Queen hypothesis in a field survey across the distributional range by assessing the frequency and intensity of pre-dispersal seed predation by dipteran larvae in over 1700 capitula and by the level of interspecific plant-plant competition (i.e. density of vascular and non-vascular plant species and vascular plant species richness) in over 700 20x20cm vegetation plots surrounding the target plants. Though significant associations in some of the measured traits were found, overall the biotic pressure differed neither between the cytotypes nor followed any spatial trend. These patterns were clearly driven by considerable between-population variation across all measured traits. Consequently, the Red Queen hypothesis cannot explain the geographical parthenogenesis in *H. alpinum*.

Questions 2 & 3

Given the allopatric distribution of the cytotypes, we supposed that the differences in ploidy level might affect the species' eco-physiological response to the local biotic and abiotic conditions. In order to address this hypothesis, I estimated the specific leaf area, foliar carbon (C), nitrogen (N) contents, and their stable isotope compositions in more than 200 plants sampled in 27 populations across the species range. I assessed within- and between cytotypes variation in these traits and their putative associations with the biotic (density and species richness of co-occurring vegetation) and abiotic environment (temperature, precipitation and solar irradiance during the growing season derived from WorldClim2 model). The cytotypes did not significantly differ in the measured traits, but diploids exhibited significantly higher foliar N content. Importantly, the effects of the latitudinal and altitudinal position of populations exceeded between-cytotype differences. Accordingly, Scandinavian populations were considerably distinct from continental European populations independent of the cytotype across all measured traits.

Question 4

Baker’s Law proposes that the advantage of uniparental reproduction enables self-compatible plants to establish a new population in the absence of pollinators and mating partners, i.e. with one individual only. In *H. alpinum* sexual diploids exhibit a strict self-incompatibility and thus are fully dependent on pollinators and on the availability of a mating partner for successful seed production. In contrast, closely related triploids of *H. alpinum* exhibit diplosporous apomixis, i.e. they form the endosperm and embryo in complete independence on pollinators and mating partners. Such differences in pollinator / mating partner dependence between sexuals and apomicts, i.e. between biparental and uniparental reproduction, has been suggested as one of the most crucial hypotheses underlying geographical parthenogenesis (Hörandl 2006). Surprisingly, evaluations of reproductive assurance in natural populations of autonomous apomicts have received almost no attention so far. For this reason, we firstly compared the level of sexual *versus* apomictic reproduction of diploid and triploid plants of *H. alpinum* across its distributional range. Secondly, we compared the potential and realized seed sets between both cytotypes under natural and pollination limited greenhouse conditions. The flow cytometric screenings confirmed obligate sexuality and obligate autonomous apomixis in diploids and triploids, respectively. The cytotypes did not differ in the total number of florets (ovules) per capitulum, i.e. potential reproductive assurance, in the field. The same applied to the realized reproductive assurance. The picture, however, changed dramatically in the greenhouse experiment. The diploids’ realized reproductive assurance, i.e. the proportion of well-developed seeds, dropped down to 3% whereas that of the triploids reached around 78%. These results provided a firm evidence that obligate autonomous apomictic reproduction encompasses a more stable and predictable seed production when compared to sexual reproduction, which might be of great importance considering the uncertainty of pollinator activity and behavior.

Therefore, these patterns might provide apomicts with increased colonization potential, which could tentatively explain the strong geographic parthenogenesis pattern observed in this species.

Question 5

Triploid apomicts are wider distributed than diploid sexual plants of *H. alpinum* and likely replaced extinct diploid lineages in parts of the range. Consequently, we proposed that the cytotypes could differ in dispersal and germination abilities with triploids being characterized by superior colonization abilities. We examined several seed characteristics (seed weight, seed length, seed width, pappus length and width, ploidy) as well as terminal velocity and germination of 970 individual seeds collected from 228 mother plants sampled in 30 populations across the species' range. The cytotypes did not differ in any of the measured traits but triploid seeds were wider. Due to high interpopulation variation, the differences in terminal velocity between the cytotypes were only significant if the population level was not considered, yielding in significantly greater terminal velocity of diploids, i.e. dispersal on shorter distance. Importantly, though the cytotypes did not differ in seed viability, the germination probability was higher in diploids compared to triploids, indicating higher seed dormancy of apomictically derived seeds compared to sexually derived seeds. We concluded that the cytotypes of *H. alpinum* follow partly opposite dispersal-germination strategies. Though triploid seeds are putatively further dispersed by wind, the probability of germination of dispersed seed was higher in diploids. The higher dormancy in triploids compensated the lower germination rate, which might enables the triploids to form a seed bank. Therefore, the wider distribution of triploids cannot thoroughly be explained by better dispersal and germination abilities.

Questions 6 & 7

I tested the differences in the exploitation of niches by sexual diploid and apomictic triploid plants of *H. alpinum*, i.e. frozen niche-variation hypothesis *versus* general-purpose genotype hypothesis, by assessing the phenotypic plasticity of (i) germination and (ii) vegetative and reproductive traits.

(i) Plasticity in germination

The retreat of the ice sheet covering Europe during the last glacial maximum (LGM) enabled those plants, which previously survived in refugia, to access deglaciated habitats, expanding the plants' distributional range. In the course of such range expansions, plants faced a series of spatial and temporal variation in environments. Given that the mortality rates of plants are the highest at germination or seedling stages (Hoya et al. 2007), it is of great importance to germinate at the right time in a spatially and temporally varying environment. Consequently, a constant germination, which is more or less independent of the environmental conditions, i.e. germination robustness (Richards, Bossdorf, Muth, Gurevitch,Pigliucci 2006), could be of great advantage in an unpredictable environment. Furthermore, species might bypass seedbank reduction in the population following germination under unfavorable environmental conditions (e.g. insufficient rainfall, low temperatures) by dormancy. Dormancy can be broken when seeds are exposed to specific conditions (temperature, moisture, light or their interactions).

In order to test the germination rate and speed under varying environmental conditions, I performed a germination experiment with sexually derived diploid and apomictically derived triploid seeds of *H. alpinum* under two different temperature regimes. Apart from addressing the germination robustness, we assessed the differences in the plasticity of germination rate and speed between the cytotypes and within the triploids. More specifically, I investigated whether apomictic lineages behave in accordance with the general-purpose genotype

(Parker Jr, Selander, Hudson,Lester 1977) or rather in accordance with the frozen niche-variation hypothesis (Vrijenhoek 1979; Vrijenhoek 1984).

Given the allopatric distribution of *Hieracium alpinum*'s cytotypes, I hypothesized that apomictically derived seeds should have an overall higher germination rate than sexually derived seeds across the two temperature regimes. I assumed that apomictically derived seeds should be less sensitive to the temperature treatment, i.e. show a flatter response and smaller plasticity in germination rate and speed when compared to sexually derived seeds. I particulary expected such a response in the widespread clonal lineage, which should show a general-purpose genotype behavior. If, however, apomictic genotypes' responses (slopes) are similar to those of the sexual genotypes, apomictically derived seeds might show frozen niche-variation behavior.

At each sampling locality, we selected 30 plants, when possible, with fully open but complete and visually non-damaged capitula with ripe achenes for seed set assessment. Sampling was conducted in two crossed-over linear transects (each with ± the same number of plants) and with 2 m as a minimum distance between plants. Collected capitula were stored in small paper bags, after sampling, transferred to silica gel, and stored in the fridge until the start of the experiment. Due to insufficient sample size and / or quality of samples, we revisited four populations sampled in 2014 and performed additional sampling of four new populations. In December 2015, the germination experiment was carried out at the Institute of Botany in Průhonice, Czech Republic. I chose 60 well developed and, visually, not damaged seeds (i.e. achenes) of five seed families (i.e. seed mother plant) per population (i.e. 300 seeds per population), when possible. The final experiment encompassed 8682 seeds originated from 184 seed families sampled in 32 populations. Additionally, we included seeds from six populations, which were sampled in two consecutive years. Corresponding to two temperature treatments (warm: 20 °C / 10 °C, cold: 12° C / 5 °C), seeds were divided into two groups and germinated on moistened filter paper on glass beads in water-filled Petri dishes randomly placed in germination chambers (Thermoforma 3, Model 3744, Thermo Fisher) at 12 h of daylight / 12 h of darkness with a cool daylight light source (920 Lux, LT 15 W T8/865, NARVA). I recorded germination success bi-daily over a period of 57 days. To ensure randomness among treatments and populations, the horizontal and vertical positions of Petri dishes inside the germination chambers were shifted bi-daily. I removed emerged seedlings and placed them on planting trays in the greenhouse for further experiment (see below). In order to describe the germination behavior of *H. alpinum*'s seeds under different environmental conditions, I focused on the following parameters, which were calculated seperately per seed family and treatment.

I used the cumulative proportion of germinated seeds by the end of the experiment to determine the germination success (i.e. final germination percentage; FGP). The germination speed was defined by the germination rate index (GRI): $GRI = G_1/1 + G_2/2 + G_x/x$, where G is the germination percentage at each alternate observation day and 1, 2, x correspond to the observation day (Esechie 1994). However, a considerably high number of seeds did not germinate until the end of the experiment, suggesting putative dormancy of those seeds. Consequently, I tested the remaining seeds for viability by gently squeezing. I considered soft seeds as not viable and hard seeds as viable, i.e. dormant. Subsequently, I calculated the proportional seed viability using the following equation: Seed viability [%] = $100*(\sum$ germinated seeds + $\sum$ dormant seeds) / $\sum$ sown seeds. In order to account for dormancy, I calculated the dormancy index as follows: Dormancy index = 1-(germination probability / seed viability) (Offord, McKensy,Cuneo 2004).

In order to assess the germination sensitivity, I calculated the relative plasticity index (RPI) on seed family level estimating the amount of response of the seeds in FGP and GRI to the temperature treatment. Therefore, the absolute difference between the parameter value of the cold and the parameter value of the warm treatment was divided by the mean of the two referring values. This index takes values between 0 (no plasticity) and 1 (high plasticity). Additionally, I calculated the reaction norms (RN) for the same parameters, at the seed family level by deducting the seed family's value in the cold treatment from the seed family's value in the warm treatment. In contrast to the RPI, this approach allows conclusions about the level and direction of response to the treatment.

(ii) Plasticity of vegetative and reproductive traits

Given the fact that *H. alpinum* occurs along a considerable latitudinal gradient (43°-73°N), encompassing subarctic and alpine habitats, plants are exposed to significantly different macroclimatic conditions along this gradient (Billings and Mooney 1968), such as the length of the day during the growing season, amount of precipitation, the intensity of solar radiation and diurnal temperature variation. Based upon this, I expected different responses to different environmental conditions (Bauert 1996) between sexual genotypes and apomictic clonal lineages, as well as between different apomictic clonal lineages. The aim of this experiment was to assess whether the apomictic genotypes follow the general-purpose genotype (Parker Jr, Selander, Hudson,Lester 1977) or rather the frozen niche-variation behavior (Vrijenhoek 1979; Vrijenhoek 1984).

As mentioned above, after the germination experiment, I transferred the emerged seedlings into seedlings trays in which they were allowed to grow under controlled environmental conditions in the greenhouse for approximately one to two months (December 2015 – January 2016). Subsequently, we repotted the survived seedlings into 3dc pots filled with a 4:2:1 mixture of peatbog : garden compost : siliceous gravel (1-2mm grainsize). Prior to starting the plasticity experiment, the repotted plants were allowed to grow for a period of approximately three weeks under the same conditions. Out of these plants, I selected, when possible, 16 plants per seed family. Plants were selected in a manner to ensure similar size of all plants. The total number of plants being part of the experiment was more than 850 originated from 57 seed families sampled in 22 populations across the species range.

I exposed the repotted plants to four different environments following a 2×2 full factorial design (Figure 6) with two different day lengths (long [20h of light / 4h of dark] *versus* short [16h of light / 8h of dark], respectively) and two water regimes (wet *versus* dry; Figure 6). Plants were either watered every 2-3 days (wet treatment) or 4-6 days (dry treatment). Importantly, any deviations from the water treatment (e.g. more frequent because of drought in summer months) were noted. Plants were arranged within the day length treatment according to the water treatment in block wise manner. In order to minimalize edge effects and to randomize the plants, these blocks were shifted bi-weekly in a clockwise manner within the day length treatment. In order to eliminate the effects of the naturally increasing day length in spring, the day length treatment was terminated at the date of the last measurements of the vegetative traits.

Figure 6. Short day treatment as a part of the plasticity experiment with diploid and triploid *Hieracium alpinum* in the greenhouse in Průhonice, Czech Republic (March 2016). The plants were exposed to two water treatments (wet *versus* dry), which were arranged into four block per each of the light treatments.

We measured the following vegetative traits: number of rosette leaves, length and width of longest leaf and number of rosettes. These traits were measured at the beginning of the experiment (beginning of March 2016) to use this parameter as a proxy for the initial biomass. Subsequently, the same traits were measured twice (end of March and end of April 2016), respectively. Considering the leaves of *H. alpinum* exhibiting a more or less ellipsoid shape, I calculated the proxy of the leaf area for all three measuring points following the formula for the area estimation of an ellipse: $0.5\times\text{length} \times 0.5\times\text{width} \times \pi$. Based upon the measuring points, I calculated the relative growth rate (RGR) for selective vegetative traits following Grotkopp et al. (2002): $\text{RGR} = (\log_e \text{W2} - \log_e \text{W1}) / (\text{t2}-\text{t1})$. Hereby, $\log_e$ W1 represents the natural logarithm of the vegetative traits one month after the start of the experiment and W2 after two months, respectively. The denominator represents the correspondent time span between measurements in days. Additionally, at the end of the experiment period assigned to the vegetative growth, i.e. after two months, I collected the largest leaf from each plant and estimated the wet and dry weight (to estimate the leaf dry matter content; LDMC), the leaf area, the specific leaf area (SLA) and the hairiness, i.e. number of hairs per cm².

The plasticity experiment lasted for eight months (from March to October 2016). Over this period, I recorded several reproductive traits on the day of flowering. These traits included the first day of flower opening, the diameter of capitulum (measured as the width of the part of the capitula encompassing the receptacle and ovules enclosed by involucral bracts), number of stems, the height of the stem with flowering capitulum, number of stem leaves, length and width of longest stem leaf of referring stem, as well as the length of longest hair of this stem leaf. At the seed ripening stage, I collected the capitula. Capitula were stored in paper bags until the total number of well developed and not well developed seeds per capitulum, respectively, were counted.

Due to time constrains, the results of both germination and plasticity experiment have not yet been completely evaluated and interpreted. Therefore, the results of these two experiments are not shown in the framework of the present thesis, although, in the case of the germination experiment, the data have been already analyzed and for the plasticity experiment the data are ready for statistical analyses.

Question 8

The colonization history of *H. alpinum* after the retreat of the glaciers possibly shaped the current cyto-geographic pattern. Based on our previous but still unpublished results after auto-polyploidization the newly formed triploids likely replaced the diploids, i.e. the triploids are descendants of extinct diploid lineages, which occurred outside of the current diploid range (Mráz et al. 2009). Assessments of the intraspecific phylogeny in a spatial-temporal context represents a powerful method to reveal which genotypes of *H. alpinum* were involved in the colonization of previously glaciated areas. In order to tackle this, I estimated the cpDNA diversity of diploid and triploid *H. alpinum* plants sampled across the species range and used a target enrichment approach. Both approaches will potentially allow me to (i) get an insight into the ancestral and derived genotypes of *H. alpinum*, especially in respect of reproduction mode (sexual *versus* apomictic); (ii) date the age of the genotypes; (iii) compare the level of heterozygosity between sexual and apomictic genotypes and between apomictic genotypes; and, (iv) infer the proportion of nonsynonymous and synonymous mutations in coding regions between sexual and apomictic genotypes.

I evaluated the cpDNA diversity of more than 550 plants using three cpDNA microsatellite loci (*petA-psbJ*, *rsp16- trnQ*, *ndhF-rpl32R*). I manually checked all these sequences and made an alignment which consisted of 2608bp long sequences per accession using MEGA software (Kumar, Stecher,Tamura 2016). Subsequently, the alignment was used to create a haplotype network (Forster et al. 2001). Phylogenetic analyses of the alignment were conducted in MrBayes (Ronquist et al. 2012). My preliminary results revealed, that the cpDNA data are in high agreement with the data by P. Mráz (unpubl.) based on more than 90 solely polymorphic AFLP markers obtained from 520 samples.

Based on the position in the haplotype network, I selected 30 samples (sexuals and apomicts, respectively), which were subsequently used for target enrichment approach (Mandel et al. 2014). Using the single copy nuclear genes obtained from this approach, I will infer the intraspecific phylogeny of *H. alpinum*. Similarly to two previous topics, the data have been only partially analysed and therefore the results are not presented in this thesis.

In the following second main part of the thesis, I will present the detailed outcomes of partial studies focused on the questions 1-5 which have been prepared in the form of manuscripts. The status of the manuscripts (published, resubmitted) is stated at the beginning of each paper.

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PAPER I

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Photo: Capitulum of *Hieracium alpinum* with seed feeding larvae of Dipteran fly. (Photo credit: P. Mráz)

The Red Queen hypothesis and geographical parthenogenesis in the alpine hawkweed *Hieracium alpinum* (Asteraceae)

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Abstract

The Red Queen hypothesis (RQH) suggests that sexuals have an evolutionary advantage in habitats with many biotic interactions (typically in southern latitudes) while asexuals are more frequent in habitats with harsher climate and consequently lowered biotic pressure (typically in northern latitudes/higher altitudes). The biotic interactions are thus considered to be a possible driver of differential geographical distributions often observed between closely related sexuals and asexuals: a pattern referred to as geographical parthenogenesis (GP). We tested the RQH in *Hieracium alpinum* L., a vascular plant species exhibiting a striking pattern of GP, by assessing the frequency and intensity with which seed predator specialists occur and the level of interspecific plant–plant competition expressed through both the surrounding vegetation density and the species richness across its distributional range. Furthermore, we tested whether there were negative associations between intra-population genetic diversity, as assessed by amplified fragment length polymorphism markers, and incidences of seed predation. Our results indicate no significant differences in frequency and intensity of seed predation nor in the level of interspecific plant–plant interactions between diploid sexual and triploid asexual populations. Furthermore, there were no consistent latitudinal and/or altitudinal patterns for the assessed parameters. Contrary to our expectations, there were either positive or non-significant associations between genetic diversity and the level of seed predation. To conclude, the RQH cannot thoroughly explain the GP in *H. alpinum*, and other alternative scenarios should be considered and tested.

Keywords: apomixis, Diptera, frequency dependent selection, geographical parthenogenesis, *Hieracium*, plant–insect interaction, plant–plant interactions, polyploidy, pre-dispersal seed predation, Red Queen, spatial gradients

Introduction

The term geographical parthenogenesis (henceforth GP) describes different geographic patterns between sexually and asexually reproducing organisms within one species or between closely related species (Vandel, 1928). According to GP, asexual organisms, as compared to sexual ones, tend to have larger distributional ranges, which are often shifted to higher latitudes or altitudes (Ghiselin, 1974; Bierzychudek, 1985; Asker & Jerling, 1992). Furthermore, asexuals tend to occur more frequently in marginal and extreme habitats (previously glaciated areas, deserts) than sexuals do (cf. Cuellar, 1977; Bierzychudek, 1985; Peck, Yearsley & Waxman, 1998; Kearney, 2005; Hörandl, 2006; Silvertown, 2008). Thus, several non-exclusive hypotheses have been proposed to explain these patterns.

In principle, asexuals are thought to have an advantage over sexuals in colonizing new territories in several regards: (1) the absence of negative effects of inbreeding depression during colonization/recolonization events (metapopulation hypothesis; Bierzychudek, 1985; Haag & Ebert, 2004); (2) more efficient exploitation of ecological niches due to either superior specialization (frozen niche variation hypothesis; Vrijenhoek, 1984; Vrijenhoek & Parker, 2009) or superior generalization (general-purpose genotype hypothesis; Parker et al., 1977); (3) the independence of mating partner(s) (Baker's Law; Baker, 1955); or (4) putatively lower pressure of pests and competitors in extreme habitats and higher latitudes [Red Queen hypothesis (RQH); Van Valen, 1973]. The RQH purports that sexual reproduction, although twice as costly as asexual reproduction (Bürger, 1999; Lively, 2010), is advantageous in the presence of co-evolving pests, parasites and competitors. In this 'race of arms' (Ebert & Hamilton, 1996), it is therefore important to produce genetically variable progeny capable of responding faster to selection and thus ultimately to reduce the risk of infection/extinction (Bürger, 1999; Burt, 2000; De Kovel & De Jong, 2001; Butlin, 2002). Sexually reproducing organisms should therefore have an advantage over asexuals in changing environments with high biotic pressure (Solé et al., 1999). In contrast, asexuals should have an advantage at species margins and more extreme habitats with presumably fewer biotic interactions (Asker & Jerling, 1992).

Accordingly, the putative role of the RQH in the GP patterns stems from a second premise: geographical clines in biotic interactions. These are typically lowered at higher latitudes and altitudes. It can consequently be expected that asexuals will flourish in these regions (Glesener & Tilman, 1978; Bell, 1982; Louda, 1982; Molau, Eriksen & Knudsen, 1989; Asker & Jerling, 1992; Hodkinson, 2005; but see Scheidel, Röhl & Bruelheide, 2003). Based on these assumptions, it has been hypothesized (Verhoeven & Biere, 2013 and references therein) that biotic interactions might have triggered the differential distribution of sexual and asexual plants across latitudinal gradients. Indeed, GP patterns have been observed in many plant species (Bierzychudek, 1985; Asker & Jerling, 1992), but the underlying factors, including the RQH, are still debated. Hörandl (2006) stated that, '[…] understanding the impact of biotic interactions on geographical distributions of apomictic *versus* sexual plants is currently limited'. To the best of our knowledge, research have preferred to study the performance of sexual *versus* asexual plant populations in terms of herbivore attack or pathogen infection, rather than using the RQH as their main approach (Burt & Bell, 1991; Kelley, 1993; Kelley & Shykoff, 1994; Chou, Chiang & Chiang, 2000; but see Espiau et al., 1997; Verhoeven & Biere, 2013). This is emphasized by a review on RQH models by Lively (2010) who referred mainly to theoretical models or studies in the animal kingdom (but see Thrall, Burdon & Bever, 2002). Given the extreme scarcity of such studies in the plant kingdom, more empirical studies need to be performed in order to gain deeper insight into the relationships between GP in plants and biotic interactions. Studies also need to focus on similar species with comparable habitats and life history along large latitudinal and/or altitudinal gradient (Johnson & Rasmann, 2011; Verhoeven & Biere, 2013). A plant's fitness can be altered remarkably through its interaction with other organisms. Pre-dispersal seed predation by Dipteran larvae in Asteraceae, to give but one example, can strongly influence the overall success of reproduction (Sárospataki, 1999; Fenner et al., 2002); this can subsequently have considerable consequences on seed production and, in turn, on population dynamics and colonization success (Pickering, 2009 and references therein). Similarly, many studies have demonstrated the negative effects of increase in surrounding vegetation density (Goldberg & Werner, 1983; Gibson et al., 1999; Freckleton & Watkinson, 2000), and surrounding species richness, on reproductive output (seed mass and number) and biomass production (Gross & Werner, 1982; Foster, 1999; Bullock, 2000; Herben et al., 2003; Bell, Karron & Mitchell, 2005; Kikvidze et al., 2006; Partzsch & Bachmann, 2011).

In our study, we tested whether selected biotic interactions and their latitudinal and altitudinal patterns might be involved in the striking distributional pattern observed between sexual and asexual forms of alpine hawkweed, *Hieracium alpinum* L. (Asteraceae; Mráz, Chrtek & Šingliarová, 2009). The interactions chosen here were frequency and predation intensity of specialized seed-feeding Diptera, and intensity/complexity of interspecific plant competition. In *H. alpinum*, sexual, strictly self-incompatible diploid plants occur in a small isolated area on the south-eastern margin of the species range (the Eastern and Southern Carpathians). Asexual triploid apomictic plants occupy the remaining and much larger part of the range spreading from the Balkans to the arctic parts of Europe (Fig. 1; Mráz et al., 2009). We hypothesize that if the RQH is involved in this striking GP pattern, then higher biotic pressure (i.e. higher frequency and intensity of seed herbivores and higher interspecific plant competition) can be expected in the range of sexuals when compared with the range of asexuals.

Given the strong association between polyploidy and apomixis in plants (Asker & Jerling, 1992), we are aware that significant differences in the biotic interactions between sexuals and asexuals may also be caused by differences in ploidy level. In fact, polyploidy might trigger important changes in plant morphology and plant physiology, which might, in turn, influence biotic interactions (Nuismer & Thompson, 2001; Thompson, Nuismer & Merg, 2004; Comai, 2005; te Beest et al., 2011; Münzbergová, Skuhrovec & Maršík, 2015). Regardless, when non-significant or even opposite trends are observed (i.e. higher biotic pressure in triploid asexuals), it could be due to the fact that biotic interactions are probably not a primary cause of the GP pattern observed in *H. alpinum*. In the event that latitudinal gradients in biotic interactions are absent, one of the key assumptions of the RQH will not be fulfilled (Verhoeven & Biere, 2013). Because, genetically, more diverse populations are thought to be less intensely parasitized due to higher incidence of rare host genotypes (Siemens & Roy, 2005), we also tested for putatively negative associations between the level of intra-population genetic variation, as assessed by amplified fragment length polymorphism (AFLP) markers, and the frequency and intensity of seed herbivory.

Material and Methods

Study species

Hieracium alpinum is an herbaceous, arctic-alpine, insect-pollinated species that grows in tundra habitats from the mountains in Central and Eastern Europe in the south to Greenland and Northwest Russia in the north. As its seeds contain pappus, it is able to disperse on long distances (Eriksson, 1992). The species consists of two cytotypes, a diploid ($2n = 2\times = 18$) and a triploid ($2n = 3\times = 27$), with a strong allopatric distribution (Mráz et al., 2009). Importantly, the cytotypes differ in their mode of reproduction. Diploid plants reproduce strictly sexually (sporophytic self-incompatibility) and produce copious amounts of homogeneously sized pollen, whereas the triploids are diplosporous apomicts characterized by parthenogenetic development of unreduced egg cells and typically do not produce pollen

(Rosenberg, 1927; Gentscheff & Gustafsson, 1940; Chrtek, 1997; Mráz, 2003; Mráz et al., 2009). *Hieracium alpinum*'s vegetative reproduction is confined to the production of accessory rosettes, which develop from hypocotyl meristems at the base of main rosette. Former investigations using AFLP markers, allozymes, internal transcribed spacers (ITS1 and ITS2) and three cpDNA loci have revealed that triploid plants are of autopolyploid origin (P. Mráz, P. Zdvořák, M. Hartmann, M. Štefánek and J. Chrtek, unpubl. data). Furthermore, one apomictic clone is remarkably present; it occurs nearly solely in Scandinavia and very frequently in the Central and Western Alps (P. Mráz, M. Hartmann, P. Choler, T. Fér, J. Chrtek, P. Taberlet, unpubl. data).

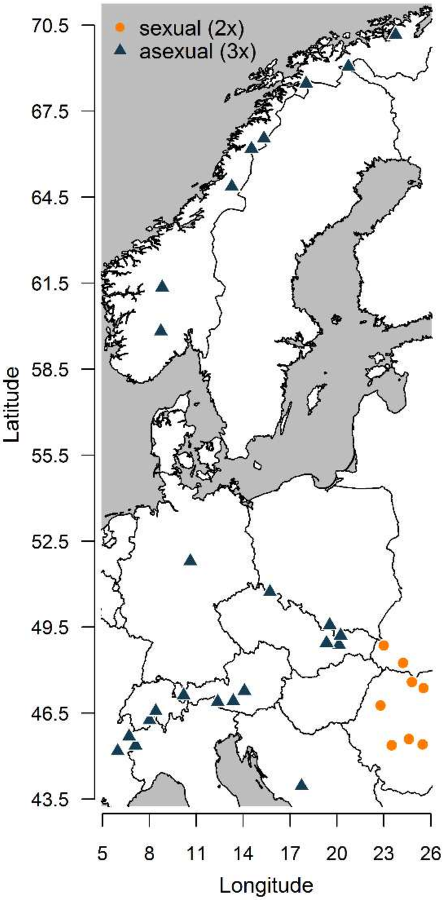

Figure 1. Locations of *H. alpinum* sampling sites. Symbols correspond to the mode of reproduction (ploidy level).

Sampling

In order to test the RQH, we visited 29 populations representatively covering geographic ranges of both diploid (the Eastern and Southern Carpathians) and triploid (the Alps, the Balkans, Scandinavia, the Sudetes mountain range and the Western Carpathians; Supporting Information, Table S1; Fig. 1) cytotypes in the summer of 2014. In the subsequent year, we performed additional sampling of four new populations and revisited four populations sampled in 2014 (Supporting Information, Table S1).

At each site, plants with a 2-m minimum distance between them were selected. We performed sampling in two different manners, where possible:

(1) In order to determine the frequency of seed predation by tephritid and anthomyiid larvae, one closed capitulum ('flower head') per plant at the stage immediately preceding its opening was randomly sampled from each of 100 plants per site (henceforth 'random sampling', Supporting Information, Table S1).

(2) In order to determine the level of plant–plant interactions, various species traits and mode of reproduction of *H. alpinum*, we conducted targeted sampling. At each site, we selected 30 plants. For seed set assessment in two crossed-over linear transects (each with ± the same number of plants), each optically undamaged capitula with ripe achenes. plant sampled had a fully open but complete and optically undamaged capitula with ripe achenes. A photography of each plant and its surrounding vegetation within a square of the size 20 × 20 cm was captured in order to estimate the co-occurring vascular plant species within the plot and vegetation density (Supporting Information, Fig. S1). In addition, we also checked for visible signs of leaf rust infection caused by *Puccinia hieracii* (Röhl.) H. Mart. However, because the overall frequency of this parasitic fungus was very low, found on 22 of 820 plants and with no geographical pattern, these data were not further statistically analysed. Although this sampling strategy targeted the plants with fully developed, undamaged and not aborted capitula, we found a relatively high number of capitula infested by tephritid and anthomyiid seed-feeding larvae during seed set assessment. We subsequently inferred the level seed predators' infestation also for this targeted sampling.

Assessment of biotic interaction

Collected capitula for seed predation assessment were stored in small, closed paper bags and either checked immediately upon sampling or later in the laboratory. Some were transferred to silica gel and kept in the refrigerator until seed predation estimation could be conducted. Seed predation per capitulum was estimated in three different ways: (1) presence/absence of seed-feeding larvae (Supporting Information, Fig. S2A–D); (2) their total number per capitulum; and (3) as a trace(s) of seed predation. We consider either visually damaged ovules/seeds by larvae or hole(s) in the bottom of a capitulum caused by larvae feeding inside the capitulum as trace(s) of seed predation (Supporting Information, Fig. S2B). Adult tephritid flies (Supporting Information, Fig. S2A; Diptera: Tephritidae; Merz, 1994), *Campiglossa guttella* (Rondani, 1870) were determined; a sole adult specimen of *Noeeta pupillata* (Fallén, 1814) was found only at one site. We also found the seed-feeding larvae of an anthomyiid fly species molecularly determined to be *Heterostylodes obscura* (Macquart, 1935) (Diptera: Anthomyiidae; L. Mazzon, M. Colombo, pers. comm.) at several sites.

Co-occurring plant species richness, considered a proxy for the complexity of interspecific competition, was assessed within each 20 × 20 cm plot according to the photographs and list of vascular plants collected in the field (Supporting Information, Fig. S1). Since accurate determination of all co-occurring vascular plant taxa was not always possible (mostly representatives of Poaceae and Cyperaceae in vegetative stage), we inferred only the total number of co-occurring vascular taxa (‘species richness’) in each plot. Vegetation cover, used as a proxy for intraspecific competition, was estimated from the photographs and was expressed as a percentage for three different layers separately: E – total vegetation cover, E0 – cover of moss and lichen layer, E1 – cover of herb and dwarf shrub layer.

Ploidy level and reproduction mode analyses

Five seeds, representing on average 12% of well-developed seeds per mother plant (P. Mráz, P. Zdvořák, M. Hartmann, M. Štefánek and J. Chrtek, unpubl. data), and mostly 30 mother plants per population were analysed by flow cytometry to assess their reproduction pathways, that is sexual or asexual. In order to check for the presence of residual sexuality in apomictic triploids, we selected 43 plants, from which nearly 25% of the total seed set per plant was analysed. Cytometrical discrimination between sexual and asexual reproduction modes in *Hieracium* s.str. is very straightforward (cf. Matzk, Meister & Schubert, 2000): due to double fertilization, sexually derived seeds have a ratio of the embryonic ploidy to the corresponding endospermic ploidy equal to 1.5 (i.e. the embryo is diploid and the endosperm triploid). In apomictically derived seeds, this ratio is always 2 (i.e. the embryo is triploid and the endosperm is hexaploid) because of their mitotically produced embryo sac (see above). The samples were prepared using a two-step procedure (Otto, 1990; Doležel & Göhde, 1995) with co-chopped leaves of *Bellis perennis* as the internal standard, 4′,6-diamino-2-phenylindole as a fluorescent stain and Partec Cyflow instrument equipped with an HBO lamp as the analyser of ploidy level. The ploidy levels of embryos and endosperms were determined as relative positions (i.e. as peaks) to the peak corresponding to the internal standard’s nuclei.

Intra-population genetic variation

The intra-population genetic variation of the selected populations involved in this study was assessed using AFLPs within a project focused on the overall phylogeographic pattern of *H. alpinum* in its whole range (P. Mráz, P. Zdvořák, M. Hartmann, M. Štefánek and J. Chrtek, unpubl. data). Therefore, at this point, we will briefly outline only the methods and evaluation of molecular data used. We applied the same DNA extraction, digestion–ligation and amplification protocols as given in Mráz et al. (2007) with two selective primer combinations: EcoRI -AGG /MseI-CTG and EcoRI -ATG / MseI-CTG . Intra-population variation was characterized as genotype/clone diversity and mean pairwise Jaccard distance among five randomly sampled individuals per population. The mean pairwise Jaccard distance as a measure of intra-population genetic variation was computed using 94 polymorphic bands recorded in 520 individual plants originating in 99 populations. The overall reproducibility of the scored bands was 99%.

Statistical analyses

First, we tested whether the sampling procedure, that is random sampling (closed capitula) *versus* targeted sampling (open capitula), had an effect on the frequency of seed predation. As expected, the test showed significantly higher incidence of seed predators in random sampling than in targeted sampling ($\chi^2 = 24.58$, $P < 0.001$; Supporting Information, Fig. S3). All further analyses were, therefore, done solely with the random sampling data set. Second, we tested whether the frequency of seed predation varied between the two sampling years at the four sites where random sampling was repeated. As this test did not show significant differences in the frequency of seed predation in any of four populations (results from χ^2 tests results not shown; Supporting Information, Fig. S4), the data from the same site from two subsequent years were merged together. To infer the intensity of seed predation, expressed as a number of seed predators per capitulum, we divided the data set into infested and not-infested capitula. From the former, we excluded those samples that showed only traces of infestation, as the actual number of predators could not be assessed accurately. We defined multiple infestation as the occurrence of more than one seed predator (irrespective of its developmental stage) per capitulum (Supporting Information, Fig. S2D). These count data were subsequently transformed into binary data (e.g. presence/absence of multiple infestation).

The effect of the mode of reproduction (sexual *versus* asexual; a fixed effect) on (1) the frequency of seed predation, (2) the complexity of plant–plant interactions (i.e. co-occurring species richness) and (3) the intensity of plant–plant interactions in vegetation plots (i.e. co-occurring vegetation density) was tested separately for each of the aforementioned explanatory variables using linear mixed-effect models (LMMs) with the identity link function for normally distributed data. We used generalized linear mixed-effect model (GLMM) and the logit link function for binomial data to assess the effect of the mode of reproduction on the intensity of seed predation. By doing so, the probability of showing multiple infestation (intensity of seed predation) was determined at the population level as a ratio between the number of capitula with multiple infestations and number of infested capitula with the presence of one seed predator only. We chose this multinomial approach because it gives more information on the size and direction of the effects of intensity of seed predation and takes the total sample size into account (Jaeger, 2008). The same approach, however, could not be applied to the model with seed predation's frequency due to the violation of the normality assumption. Instead, we calculated the overall proportion of infested capitula at the population level. Given the huge latitudinal gradient covered by our sampling, each population was assigned to one of the five regions (principally mountain ranges): the Alps, the Eastern Carpathians, the Southern Carpathians, Scandinavia and the Western Carpathians (Supporting Information, Table S1). The two isolated populations, namely H87 from the Vranica Planina and C2 from the Apuseni Mountains, were assigned to the geographically closest regions, that is to the Alps and the Eastern Carpathians, respectively. Importantly, each region and population was homogeneously composed of either sexual or asexual populations and plants, respectively (see Results). The region was included in the models as a random term assuming more similar biotic interactions among geographically related populations. The analyses were performed using lmer and glmer functions implemented in lme4 package (Bates et al., 2015) within the R environment (R Core Team, 2014), and the statistical significance of terms was assessed using a likelihood ratio test (LRT) to compare a fully fitted model to the model from which the tested term was removed. The complexity and intensity of plant–plant interactions were determined on plant level using plant–plant interaction variable as a fixed factor and the population nested within one of the five regions as a random factor. Since initial model assessments indicated strong variation within reproduction modes (Figs 2–4; Supporting Information, Table S2), we also tested for regional differences (1) in plant–herbivore interactions using general linear model (GLM) and linear model (LM) and (2) in plant–plant interactions using LMM as described above, but with region as a fixed factor.

Latitudinal and altitudinal clines of biotic interactions concerning key assumptions of the RQH (Verhoeven & Biere, 2013) were tested in the same manner outlined above. However, given huge latitudinal gradient of our sampling sites and significant negative correlation between latitude and altitude in the distribution of alpine plant communities (Arroyo et al., 2009), the altitudinal gradient was tested without Scandinavian populations, which occur at significantly lower altitudes than the populations from Central and Eastern Europe. The tests were conducted with (1) the complete data set, including sexual and asexual populations, and (2) separately for each mode of reproduction using LMM and GLMM models; the same model structure was applied as above, but with the populations' latitudinal and altitudinal positions as fixed effects.

Because our observations indicated huge interpopulation differences in frequency and intensity of seed predation (Supporting Information, Table S2), we tested whether selected plant [the size of capitulum (expressed as a number of florets per capitulum) and plant height] and habitat parameters (vascular plant richness and vegetation densities of herb and shrub layer) could explain such inter-population variation. In order to accomplish this, we performed LMM tests for the three data sets (see above) and with population means of both response (seed predation frequency and intensity). We used plant and habitat traits as explanatory variables and region as a random variable. In order to achieve normality distribution of residuals, in all models, the population average of infested capitula was square root transformed and mean vegetation densities were transformed according to Warton & Hui (2011). Accordingly, small values were added to the numerator and denominator ($\varepsilon_E = 0.01$, $\varepsilon_{E1} = 0.05$, $\varepsilon_{E0} = 0.01$) to prevent undefined values $-\infty$ and ∞.

We used LMMs to test separately for putative associations between the intra-population genetic diversity values as response variables and the population means of biotic interactions (frequency and intensity of seed predation) as explanatory variables. The region was included in the model as a random term. The tests were performed for the three data sets (see above).

Results

Geographical parthenogenesis pattern

Flow cytometrical seed screening analyses confirmed a non-overlapping distributional pattern of apomictic and sexual populations. The plants from the diploid range (Eastern and Southern Carpathians) produced the seeds with diploid embryos and triploid endosperms, accordingly sexually, while the plants from the triploid range (the Alps, Scandinavia, Sudetes, Vranica Planina and the Western Carpathians) produced the seeds with triploid embryos and hexaploid endosperms, accordingly asexually. The sexual populations occurred at significantly lower latitudes (sexual: mean ± SD 46.6 ± 1.2; apomictic: 55.8 ± 10; t-test: $P < 0.01$) and altitudes [sexual: 1833 ± 247 m, apomictic (without Scandinavia): 2158 ± 283 m; t - test: $P < 0.05$] than asexual populations, thus confirming the GP pattern.

Frequency and intensity of seed predation

The frequency of seed predation was higher in apomictic populations compared with sexual populations (sexual: 27.9 ± 30.6%; apomictic: 41.4 ± 26.4%; Fig. 2A), but this difference was only marginally significant (Table 1). At the regional level, we observed a high variation within each of studied regions (Eastern Carpathians: 25.7 ± 28%, Southern Carpathians: 30.6 ± 40.3%, Alps: 28.4 ± 9%, Scandinavia: 51.7 ± 34.2%, Western Carpathians: 39.6 ± 18.9%) and no statistically significant differences among them (Tukey test: $P > 0.05$; Fig. 3, Fig. S6A, Supporting Information, Table S2). On average, the plants from sexual populations were attacked more than twice as intensely than plants from apomictic populations (sexual: 55 ± 24.6%, apomictic: 25.8 ± 23%), but this difference was not significant either (Table 1). The sexually reproducing plants were attacked significantly more intensely than asexual plants from the Alps and Scandinavia (Tukey test: $P < 0.001$; Fig. 2B; Supporting Information, Fig. S6B, Table S2). However, the Scandinavian group also showed the highest interpopulation variation in frequency of seed predation (Fig. 3; Supporting Information, Fig. S6A, Table S2; Levene test of variance: $F = 8.8$, $P < 0.01$).

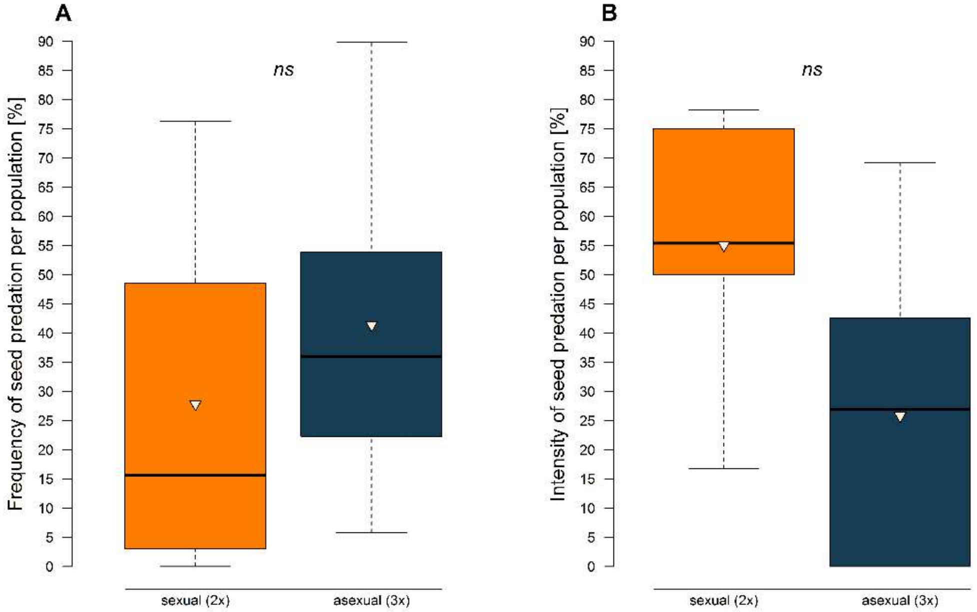

Figure 2. Boxplots of frequency (A) and intensity (B) of seed predation in sexual diploid and asexual triploid populations of *Hieracium alpinum*. Triangles indicate mean values. ns, not significant differences.

The intensity of seed predation per population was negatively correlated with the frequency of seed predation per population using the complete data set

Table 1. Results of likelihood ratio tests (χ^2 values) of LMMs and GLMM, † examining the differences in biotic interactions between populations with contrasting mode of reproduction, sexual *versus* asexual, in *Hieracium alpinum*.

Fxied and random effects / response	***Predator-plant interaction***						***Plant-plant interaction***							
	Frequency			Intensity†			Richness		E cover		E1 cover		E0 cover	
Reproduction mode	3.14	0.076	↓	3.63	0.057	↑	0.052	*NS*	0.18	*NS*	0.86	*NS*	0.96	*NS*
Region	NT			NT			1.153	*NS*	0	*NS*	0.467	*NS*	1.227	*NS*
Population	NI			NI			245.7	***	132.7	***	170.4	***	211.4	***

For seed predation variables region and for plant–plant interaction variables, population nested within region were included as random factors. The direction of arrows indicates whether sexual diploid populations reached lower (↓) or higher (↑) values when compared with asexual triploid populations. E cover, total vegetation cover; E1, herb and dwarf shrub layer cover; E0, bryophyte and lichen layer cover; richness, number of co-occurring vascular plant species (species richness); NI, not included; *NS*, not significant ($P > 0.05$); NT, not tested. ***$P < 0.001$; for marginally significant differences, exact *P*-values are given.

(GLMM: $\chi^2 = 8.93$, $P < 0.01$) and the data set with the sexual populations only (GLMM: $\chi^2 = 7.28$, $P < 0.01$). No such association was found considering the apomictic populations only (GLMM: $F = 1.09$, $P_{LRT} > 0.05$). When testing for possible associations between the frequency and intensity of seed predation and plant and habitat parameters at population level, we found that as the stem height increased, so did the frequency of multiple seed predation per capitulum (i.e. intensity) (LMM: $\chi^2 = 8.45$, $P = 0.07$); this pattern was more pronounced in asexual population than in sexual population (LMM: $\chi^2 = 10.58$, $P < 0.01$). Furthermore, we found significant positive associations between the intensity of seed predation and total vegetation cover for the complete and apomictic data sets (LMM: $\chi^2 = 5.61$, $P < 0.05$; $\chi^2 = 8.05$, $P < 0.01$) as well as mean cover of herb and shrub layer for the complete and apomictic data sets, respectively (LMM: $\chi^2 = 8.45$, $P < 0.01$; $\chi^2 = 10.58$, $P < 0.01$). The intensity of seed predation in sexual populations was negatively correlated with vascular plant species richness (LMM: $\chi^2 = 5.21$, $P < 0.05$), as well as total vegetation cover (LMM: $\chi^2 = 4.81$, $P < 0.05$). The sexual populations with larger flower heads were significantly more intensely attacked by larvae of frugivorous flies than the sexual populations with smaller flower heads (GLMM: $\chi^2 = 5.11$, $P < 0.05$).

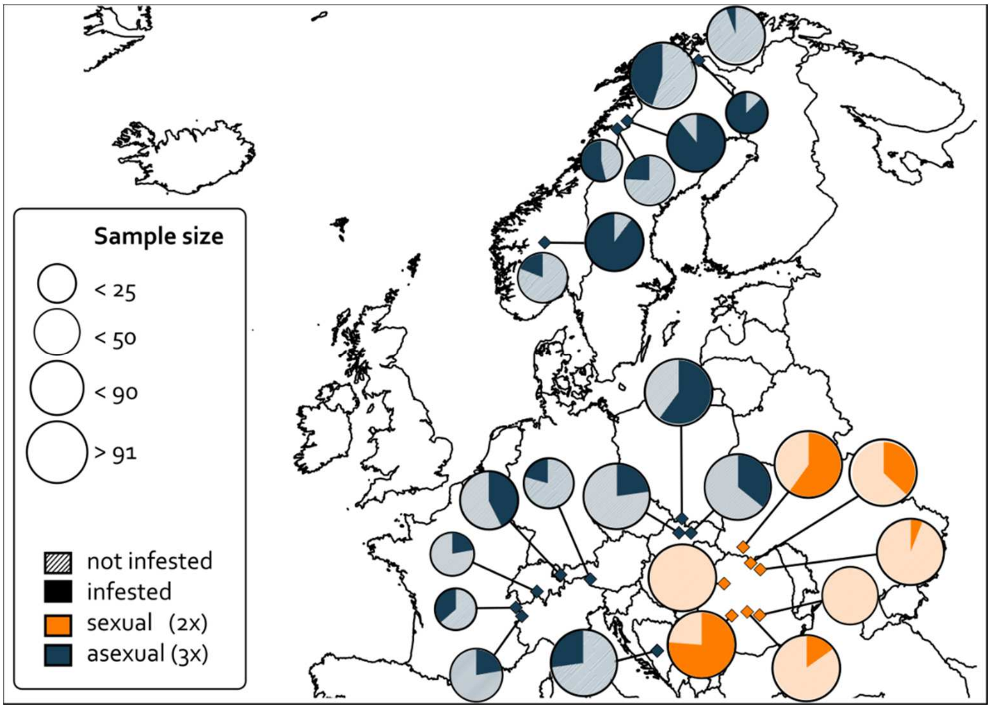

Figure 3. Map showing the frequency of seed predation per population of sexual diploid and asexual triploid *Hieracium alpinum*. Filled parts refer to infested, empty parts to not-infested capitula.

Interspecific plant-plant interactions

Sexual plants in the plots surveyed were surrounded by slightly denser vegetation in general (E layer, sexual plots: 87.6 ± 7.5%, apomictic plots: 86.1 ± 10.8%) and by denser vegetation of herb and shrub layer (E1 layer; sexual plots: 70.6 ± 12.3%, apomictic plots: 64.5 ± 15.6%), but they were less densely surrounded by bryophytes and lichens (E0 layer; sexual plots: 17.1 ± 9.8%, apomictic plots: 23.6 ± 12.3%) than asexual plants. In contrast, apomictic plants faced more diverse vascular plant communities than sexual plants (average number of species of vascular plants per plot ± SD, sexual plots: 4.32 ± 1.61, apomictic plots: 4.77 ± 1.33). All these differences, however, were only subtle and not statistically significant (Table 1, Fig. 4). The regions did not differ significantly in any of parameters except for species richness (Supporting Information, Fig. S9A–D). Plants from the Eastern Carpathians faced significantly less diverse vascular plant communities than the plants from the Alps and Southern Carpathians (Eastern Carpathians: 3.4 ± 1.3, Southern Carpathians: 5.9 ± 2, Alps: 5.4 ± 2.4, Scandinavia: 4.5 ± 1.6, Western Carpathians: 3.76 ± 1.29; Tukey test, $P < 0.05$; Supporting Information, Table S2, Fig. S9).

Across all data sets (i.e. complete, sexual, asexual), increasing co-occurring vascular plant species richness was positively correlated with total vegetation cover (LMM: $\chi^2 = 20.96$, $P < 0.001$; $\chi^2 = 3.85$, $P < 0.05$; $\chi^2 = 17.87$, $P < 0.001$) and cover by vegetation of herb and shrub layer (LMM: $\chi^2 = 21.86$, $P < 0.001$; $\chi^2 = 4.88$, $P < 0.05$; $\chi^2 = 17.33$, $P < 0.001$).

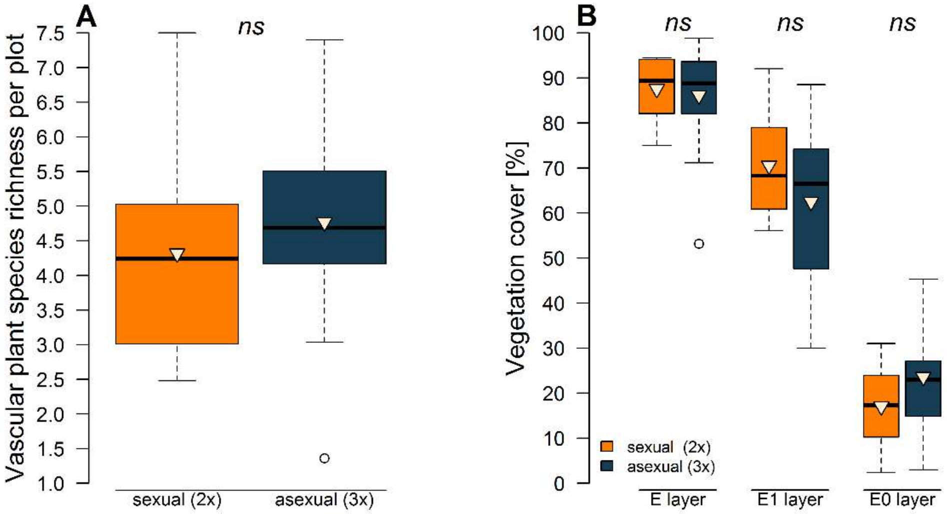

Figure 4. Boxplots of plant–plant interaction parameters in sexual diploid and asexual triploid populations of *Hieracium alpinum* observed in 20 × 20 cm vegetation plots. A, number of co-occurring vascular plant species (species richness). B, percentage of total cover by co-occurring plant species (E); percentage of cover by herb and dwarf shrub layer (E1); percentage of cover by bryophyte layer (E0). Triangles indicate mean values. ns, not significant differences.

Biotic interactions along latitudinal and altitudinal gradients

There was a marginally significant positive trend in seed predation frequency along latitude (Table 2). When considering only sexual populations, we detected a significant increase in seed predation intensity and a significant decrease in vascular plant species richness with increasing latitude (Table 2). Furthermore, the plants from the southernly situated populations faced denser vascular plant communities (E1 layer) than the plants from the populations situated northernly; the opposite trend was recorded for the bryophyte/lichen layer (E0). Such a pattern appears to have been driven mostly by asexual triploid populations (Table 2).

When testing for altitudinal gradients, one consistent pattern emerged in three data sets (see above): vascular plant species richness increased with altitude. In addition, the intensity of seed predation was positively correlated with altitude within species sexual range, while negative correlation was found between frequency of seed predation per population and altitude in population within the species asexual range (Table 2).

Intra-population genetic variation and its associations with seed herbivory

As investigations on the associations of genotype/clone diversity (results not shown) and Jaccard index with seed herbivory revealed the same pattern, we have decided to focus on the Jaccard index only. Mean intra-population genetic variation was substantially greater in sexual diploid populations (mean value of Jaccard distance per 2x population = 0.53 ± 0.10 SD) than in asexual triploid populations (mean value of Jaccard index of dissimilarity = 0.13 ± 0.14 SD; Wilcoxon test, $W = 105$, $P < 0.001$), thus corroborating the effect of reproduction on genetic variation. The lowest level of genetic diversity was recorded in apomictic populations from Scandinavia (Supporting Information, Table S2), which were either solely or to a large portion composed of the widespread clone. Populations with higher level of genetic diversity in the sexual range were infested by Tephritid flies more frequently than genetically less diverse populations ($\chi^2 = 8.34$, $P < 0.01$); however, no such patterns were found in the whole and asexual data sets, respectively ($\chi^2 = 0.9$, $P > 0.05$; $\chi^2 = 0.01$, $P > 0.05$). Mean values of intensity of seed predation per population were positively associated with intra-population genetic diversity when tested against the whole data set ($\chi^2 = 5.23$, $P < 0.05$), but not when tested against sexual and asexual data sets separately ($\chi^2 = 0.34$, $P > 0.05$; $\chi^2 = 3.34$, $P > 0.05$).

Table 2. Results of likelihood ratio tests (χ^2 values) examining relationships between spatial positions of *Hieracium alpinum* populations and the level of biotic interactions.

	Latitude			***Altitude***			
Dataset	complete	sexual	asexual	complete	sexual	asexual	Scandinavia
Seed Predation							
Frequency	2.77 0.095 ↑	0.33 *NS*	1.48 *NS*	0.12 *NS*	0.36 *NS*	**5.66** * ↓	0.00 *NS*
Intensity†	0.73 *NS*	**6.06** * ↑	0.15 *NS*	3.80	**7.24** ** ↑	0.02	NA 0.06 ↓
Plant-plant interaction							
Richness	0.04 *NS*	**5.55** * ↓	0.46 *NS*	**11.5** *** ↑	**6.39** * ↑	**8.36** ** ↑	0.03 *NS*
E cover	0.13 *NS*	0.22 *NS*	0.03 *NS*	0.51 *NS*	0.53 *NS*	1.52 *NS*	1.09 *NS*
E1 cover	**6.20** * ↓	1.62 *NS*	**4.3** * ↓	0.12 *NS*	1.10 *NS*	1.45 *NS*	0.81 *NS*
E0 cover	**8.66** ** ↑	0.93 *NS*	**6.79** ** ↑	0.03 *NS*	0.08 *NS*	0.21 *NS*	0.29 *NS*

LMM and GLMM, † were applied to three different data sets separately: complete data set (containing both sexual and asexual populations), and sexual and asexual data sets, respectively. The Scandinavian asexual populations were excluded from both complete and asexual data sets when testing for altitudinal cline and were analysed separately (see Material and Methods). The directions of statistically significant associations between biotic interaction variables and latitudinal and altitudinal positions of populations are indicated with arrows. E cover, total vegetation cover; E1, herb and dwarf shrub layer cover; E0, bryophyte and lichen layer cover; richness, number of co-occurring vascular plant species (species richness); *NS*, not significant ($P > 0.1$). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; for marginally significant differences, exact P-values are given.

Discussion

We initially assumed that if the RQH plays a role in the GP pattern of *H. alpinum*, then plants from sexual populations will experience higher biotic pressures, compared with plants from asexual populations, that is a higher frequency and intensity of seed predators and increased levels of plant–plant interactions. Moreover, the intensity of biotic interactions are thought to decrease as the populations' latitudinal and altitudinal positions increase (Asker & Jerling, 1992; Verhoeven & Biere, 2013). In spite of our sampling efforts, which included 29 populations and covered huge latitudinal and longitudinal gradients, our results are not consistent with these assumptions. Our results rather suggest high inter-population variation, which is independent of breeding system, and, to a large extent, latitudinal/altitudinal position of populations.

Frequency, intensity and spatial gradients of seed predation

In our study, we found two opposite trends in seed predation; they were, however, only marginally significant (Table 1). While asexual populations showed, on average, higher frequency of seed predation reproducing populations, the latter were attacked more intensely by seed predators. Across all populations, there were no significant latitudinal and altitudinal gradients in either seed predation frequency or intensity (Table 2). When tested separately, however, the frequency of infestation was significantly negatively correlated with altitude in the asexual range, whereas the intensity of seed infestation was significantly positively correlated with increasing altitude and latitude in sexual range (Table 2).

Concerning the frequency of seed predation, our data are aligned with the findings of the sole study focused on plant–seed herbivory interactions in plant apomictic complexes. In this study, Verhoeven & Biere (2013) found no significant association between the infestation rate and the proportion of asexual plants in populations of *Taraxacum officinale* (common dandelion). When testing for differences between two predefined groups of populations (i.e. the northern group: purely apomictic populations; the southern group: pure sexual, pure apomictic and mixed populations), Verhoeven & Biere (2013) found significantly lower infestation rates in the northern region. Concerning the intensity of seed predation in *H. alpinum*, plants from sexual populations were twice as intensely infested by larvae of Dipteran flies as plants from asexual populations. Despite the lack of significance, this implies a higher biotic pressure at the level of the individual plant, as the proportion of damaged seeds is paralleled by the number of seedfeeding larvae per capitulum (Honek & Martinkova, 2005). This pattern, however, was driven mostly by sexual populations from the Eastern Carpathians, while sexual populations from the Southern Carpathians had comparable levels of multiple infestation with those found in asexual populations (Supporting Information, Fig. S8).

More numerous studies based on animal parthenogenetic systems revealed non-consistent patterns. They have either demonstrated (Lively, 1987, 1992; Dybdahl & Lively, 1998; Kumpulainen, Grapputo & Mappes, 2004; Decaestecker et al., 2007; Morran et al., 2011; Kerstes et al., 2012) or failed to demonstrate higher pest pressures in sexual organisms than in asexual ones (Ben-Ami & Heller, 2005; Killick et al., 2008). Likewise, there are no consistent latitudinal and altitudinal patterns in seed predation in vascular plants. While some studies revealed decreasing levels of seed predation at higher latitudes (Coley & Aide, 1991; Coley & Barone, 1996) or altitudes (Louda, 1982; Randall, 1986; Molau et al., 1989), others, including two meta-analyses papers, have not (Garcia et al., 2000; Moles & Westoby, 2003; Scheidel et al., 2003; Moles et al., 2011a, b).

In our study, both frequency and intensity of seed predation varied considerably, even among geographically close populations in the same region (Fig. 3; Supporting Information, Fig. S6). This strongly suggests that this variation is dependent neither on breeding system nor on the geographical position of populations but probably on other factors. In accordance with our results, huge inter-population variation in host plant-seed predator interactions, even at a patch scale (Honek & Martinkova, 2005), has been recorded in number of studies (e.g. Garcia et al., 2000; Leimu & Syrjänen, 2002; Leimu et al., 2002; Thompson, 2005; Maron & Crone, 2006; Ågren, Ehrlén & Solbreck, 2008; Armbruster, Lee & Baldwin, 2009; Gómez et al., 2009; Vanhoenacker, Agren & Ehrlén, 2009; Verhoeven & Biere, 2013). Such huge variation might stem from the fact that the likelihood of being infested and the level of infestation depend on various biotic and abiotic factors and their often very complex interactions (Kolb, Ehrlén & Eriksson, 2007; Schemske et al., 2009). Among the most important factors are (1) size and density of host organisms (Ehrlén, 1996; Fenner et al., 2002; Weppler & Stöcklin, 2006; Arvanitis, Wiklund & Ehrlén, 2007), (2) flowering duration (Fenner et al., 2002), (3) intra-population synchrony of flowering (positive relationship: Elzinga et al., 2007, but see Fenner et al., 2002), (4) plant secondary metabolites (Castellanos & Espinosa-García, 1997; Lattanzio, Lattanzio & Cardinali, 2006) and (5) microhabitat (including co-occurring vegetation) and microclimatic conditions (Sterk & Luteijn, 1984; Verduijn, Van Dijk & Van Damme, 2004; Maron & Crone, 2006; Ågren et al., 2008; Knochel & Seastedt, 2010; von Euler, Agren & Ehrlén, 2014). Indeed, measured population characteristics (mean stem height, size of capitulum) and the habitat conditions mirrored, for example in co-occurring species, richness and density (Supporting Information, Table S2), varied markedly in our study. Accordingly, several significant associations, especially between the intensity of seed predation and plant phenotype/vegetation characteristics, have been established, although they might not necessarily express direct causal relationships. Nevertheless, some of these associations were previously confirmed in other model systems, such as the positive relationship between flower head size in Asteraceae and the frequency of multiple infestation (Straw, 1989a, b; Fenner et al., 2002).

Some authors have also stressed the importance of between-year variation in seed predation (Ehrlén, 1996; Leimu et al., 2002; von Euler et al., 2014). However, repeated observations at four sites of *H. alpinum* during two consecutive years revealed consistent patterns in agreement with the findings in *T. officinale* (Honek & Martinkova, 2005). Inter-population variation in seed predation can also be affected by the strength of host–predator specificity (Straw, 1989a, b; Crawley, 1992). Larvae of *C. guttella* and *N. pupillata* are frugivorous specialists of the flower heads of several *Hieracium* taxa, but both species can also use several other genera of Asteraceae as host organisms (White, 1988; Merz, 1994). Although *H. alpinum* is frequently the only species that can be found in the alpine belt, it may co-occur with other *Hieracium* taxa (e.g. *Hieracium halleri*, the *Hieracium piliferum* group, the *Hieracium nigrescens* group). In our study, we found these taxa at several sampling sites, but the frequency and level of infestation were fairly similar to co-occurring *H. alpinum* at all but one site. At this site (H101, Scandinavia), we observed a very high infestation rate (achieving almost 85% of sampled capitula) in *H. nigrescens* s.l., while later flowering *H. alpinum* was infested only at 45% (Supporting Information, Table S2). The co-occurrence with other *Hieracium* species with shifted phenology could thus also contribute to the observed between-population variation in flower head infestation in *H. alpinum*.

Our study did not confirm the theoretical prediction about the negative relationship between the level of infestation of parasitic flies and the population's genetic diversity, as the parasites should overcome more host genotype barriers in genetically more diverse populations (see Siemens & Roy, 2005 and references therein). On the contrary, our data revealed a significant opposite trend: the populations with higher genetic diversity (higher number of clones) were infected more frequently/intensely than genetically less diverse populations. The pattern observed in our study could be affected by (1) a lag phase between the level of parasitism and the population's genetic diversity (Siemens & Roy, 2005) considering the cyclic nature of host–parasite interactions (Ebert & Hamilton, 1996), (2) the stronger effect of a particular genotype/clone than the level of population diversity/

rarity (Strauss & Karban, 1994) or (3) stochastic processes (see above). Since we performed our assessment of genetic diversity and incidence of parasitism at a single point, we were not able to assess the possible effects of lag phase. With respect to the influence of particularly resistant clone(s)/(genotype(s), this explanation can be ruled out, as the Scandinavian populations composed of mostly one widely distributed clone were attacked by Tephritidae flies to a varying extent (Fig. 3; Supporting Information, Fig. S7). Our results thus strongly support the idea that disease/parasitic resistance in plants is a complex trait probably not caused by specific allelic combinations and interplay of environmental factors and fitness trade-offs have to be taken into account to explain host–parasite co-evolutionary dynamics (Kover & Caicedo, 2001; Neiman & Koskella, 2009).

Intensity, complexity and spatial gradients of interspecific plant-plant interactions

We did not find any differences between sexual and asexual populations in the level of interspecific plant interactions expressed in terms of co-occurring vegetation density and vascular plant species richness. The same can be said for the results reported on seed predation: both plant–plant interaction parameters varied considerably among populations (Table 1; Supporting Information, Table S2). The habitat/niche differentiations between sexuals and asexuals have previously been revealed in two apomictic plant complexes, namely in *T. officinale* (Meirmans et al., 1999) and *Ranunculus kuepferi* (Kirchheimer et al., 2016). In both cases, sexuals preferentially occurred in more stable habitats or regions with putatively higher interspecific competition, while asexual occupied more disturbed or more extreme habitats with putatively lower level of biotic interactions. While historical processes, that is anthropogenic disturbance and possibly later immigration of asexuals, could shape this pattern in *Taraxacum* (Meirmans et al., 1999), Kirchheimer et al. (2016) hypothesized that the distribution of asexual tetraploids of *R. kuepferi* was at least partly constrained by differences in competitive abilities between both cytotypes in warmer, more productive environments. However, this competitive constraint does not seem to be completely effective due to partial sympatric situation of both forms (Kirchheimer et al., 2016). This suggests that co-evolution with antagonistic competitors may not be tight enough to be advantageous for sexuals (Meirmans et al., 1999; Meirmans, 2005).

One possible explanation is that the plant–plant interactions in natural populations are far more complex than our simplistic a priori assumptions based solely on antagonistic (competitive) relationships. In fact, the balance between facilitative and competitive effects varies with the life stages and physiology of the interacting species, indirect interactions with other species and the intensity of abiotic stress (Callaway & Walker, 1997). Furthermore, this balance may also change from year to year (Herben et al., 2003). The natural complexity of plant–plant interactions may, therefore, not be grasped by an experimental approach. To our knowledge, there are only two studies comparing competitive abilities between sexual and asexual forms in plant apomictic complexes. While De Kovel & De Jong (2001) did not find differences between diploid sexuals and triploid asexual dandelions (*Taraxacum*), sexual tetraploids in *Pilosella officinarum* (Asteraceae) were better interspecific competitors, but weaker intraspecific (inter-cytotype) competitors when compared to asexual pentaploids (Sailer et al., 2014). Interestingly, this effect disappeared when artificially created sexual pentaploids were compared to apomictic pentaploids (Sailer et al., 2014), which suggests a potential effect of polyploidy dosage. Following this line of evidence, Mau et al. (2015) found niche differentiation between diploid and polyploid taxa in the genus *Boechera* (Brassicaceae), but not between sexuals and related asexuals of the same ploidy level.

Concerning spatial gradients, we found that plots of populations from higher altitudes were significantly more species rich than plots of populations from lower altitudes. With the exception of the sexual range, we did not find significantly negative association between latitude and co-occurring species richness, although the trend towards richer communities in the southern regions (the Alps, the Southern Carpathians) was obvious (see Results). Given the positive association between species richness and vegetation cover, our findings rather underline the aforementioned complexity of plant–plant interactions in our study. Nevertheless, non-significant associations between latitude and species richness are rather surprising given the huge latitudinal gradient covered by our sampling and the differences in regional species pools (Tasenkevich, 1998; Aeschimann, Rasolofo & Theurillat, 2011; Puşcaş & Choler, 2012). The pattern we found in our study could be tentatively explained by the overall low level of species richness of the alpine plant communities on acidic bedrock and small size of our plots (0.4 m^2), which was, however, adopted to track immediate plant– plant interactions around *H. alpinum* individuals. Furthermore, the density of herb layer was negatively correlated with latitude and exactly the opposite pattern was found in bryophyte layer. The latter pattern thus resulted in a non-significant trend in total vegetation density (herb and bryophyte layers together).

Conclusions

Our results do not support the hypothesis that geographical distributional patterns of sexuals and asexuals in *H. alpinum* are driven by differential antagonistic relationships with pre-dispersal seed parasites and co-occurring vegetation. In contrast, our study revealed huge variation in the assessed traits, which might be caused by the high complexity and variability of biotic and abiotic parameters and their interactions across the range of the species. To explain the causality of striking GP pattern in *H. alpinum*, other hypotheses like an advantage of higher reproductive assurance or ploidy level should be examined further. Given the scarcity of observational and experimental studies on plant apomictic complexes, more studies should be performed in different model systems to draw a more conclusive picture about the role of competition in the GP patterns in vascular plants.

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Supporting Information

Table S1. List of sexual diploid and asexual triploid populations of *Hieracium alpinum* and number of plants per population sampled for seed predation assessment.

Pop	*Locality*	*N*	*E*	*Alt. [m]*	*Date of visit*	*∑ capitula (Method)*	
						Random	*Targeted*
sexual diploid populations							
C1	RO, Rodnei Mts., the Şaua Gargalău saddle, (EC)	47.58	24.80	1770	12-Aug-14	105	56
C2	RO, Apuşeni Mts., Mt. Vlădeasa, (EC)	46.76	22.79	1788	28-Jul-15	104	30
H57	RO, Bistriţa Mts., Mt. Pietrosul Bogolin, (EC)	47.39	25.54	1693	11-Aug-14	31	59
H57	RO, Bistriţa Mts., Mt. Pietrosul Bogolin, (EC)	47.39	25.54	1693	30-Aug-15	71	27
H63	UA, Svydovets Mts., Mt. Stih, (EC)	48.25	24.23	1416	28-Jul-14	107	29
H67	UA, Skchidni Beschady Mts., Mt. Pikuji, (EC)	48.86	23.00	1357	21-Jul-14	NS	40
H73	RO, Parăng Mts., Mt. Parăngul Mic, (SC)	45.38	23.51	2099	07-Aug-14	100	46
H73	RO, Parăng Mts., Mt. Parăngul Mic, (SC)	45.38	23.51	2099	13-Aug-15	73	NS
H74	RO, Buçegi, Mts., Mt. Babele, (SC)	45.41	25.48	2120	09-Aug-14	NS	44
H74	RO, Buçegi, Mts., Mt. Babele, (SC)	45.41	25.48	2120	15-Aug-15	88	NS
H77	RO, Fagaraş Mts., Mt. Arpaşu Mic, (SC)	45.59	24.63	1944	08-Aug-14	100	38
H77	RO, Fagaraş Mts., Mt. Arpaşu Mic, (SC)	45.59	24.63	1944	14-Aug-15	15	NS
asexual triploid populations							
H110	FR, Massif de la Vanoise, Les Evettes, (AP)	45.37	7.11	2599	24-Aug-14	6	40
H110	FR, Massif de la Vanoise, Les Evettes, (AP)	45.37	7.11	2599	28-May-15	35	47
H111	FR, Massif du Beaufortain, Col du Cormet de Roselend, (AP)	45.69	6.70	2137	24-Aug-14	11	39
H114	AT, Vorarlberg, Arlbergpass, (AP)	47.12	10.19	2232	15-Aug-15	54	37
H20	AT, Defereggengebirge, Mooseralm, (AP)	46.89	12.37	2359	22-Aug-14	34	33
H26	AT, Schladminger/Wölzer Tauern, Sölkpass, (AP)	47.27	14.08	1692	21-Aug-14	NS	5
H29	FR, Massif de Belldonne, Lac du Petit Domènon, (AP)	45.16	5.95	2390	26-Aug-15	NS	9
H3	CH, Wallis, Simplonpass, (AP)	46.26	8.01	2300	13-Aug-15	NS	13
H4	CH, Wallis, Furkapass, (AP)	46.57	8.41	2457	23-Aug-14	NS	56
H4	CH, Wallis, Furkapass, (AP)	46.57	8.41	2457	30-Aug-15	27	38
H87	BA, Vranica Planina Mts, Mt. Nadkrstac, (AP)	43.96	17.74	2040	05-Aug-14	100	26

Table S1 continued

H82	CZ, Krkonoše Mts, Mt. Studniční hora, (WC)	50.73	15.71	1416	16-Aug-14	NS	11
H100	NO, Nordland, Arctic Polar Circle, (SV)	66.55	15.33	729	26-Aug-14	84	56
H101	NO, Nordland, Narvik, Gáicajávrrit (SV)	68.46	18.03	461	27-Aug-14	116	43
H102	NO, Storfjord (SV)	69.05	20.74	561	27-Aug-14	16	38
H103	NO, Finmark, Bigás, (SV)	70.16	23.75	505	28-Aug-14	52	46
H94	NO, Telemark, bellow Mt. Gaustakane, (SV)	59.82	8.73	1270	21-Aug-14	38	3
H95	NO, Øystre Slidre, Mt. Båtskardet stølane, (SV)	61.31	8.81	1097	22-Aug-14	88	43
H99	NO, Mo i Rana, Mt. Hellerfjellet, (SV)	66.2	14.5	871	25-Aug-14	33	49
S1	NO, Oppland, Vang al Vaji, (SV)	62.41	9.41	1220	23-Aug-14	NS	5
S2A	NO, Nordland, Steinfjellet, (SV)	64.87	13.26	703	24-Aug-14	NS	6
S2B	NO, Nordland, Steinfjellet, (SV)	64.87	13.27	737	24-Aug-14	NS	11
S3A	NO, Nordland, Steinfjellet, Mt. Bjørkvassklumpen, (SV)	64.87	13.28	733	24-Aug-14	13	12
S3B	NO, Nordland, nearby the Steinfjellet-tunnel , (SV)	64.86	13.25	596	24-Aug-14	NS	18
H32	SK, Západné Beskydy Mts., Mt. Babia hora, (WC)	49.57	19.53	1704	26-Jul-15	100	33
H33	SK, Nízke Tatry Mts, Mt. Chopok, (WC)	48.94	19.32	1961	10-Aug-14	101	30
H42	SK, Nízke Tatry Mts, Mt. Kráľova hoľa, (WC)	48.89	20.13	1931	09-Aug-14	103	30
H47	SK, Vysoké Tatry Mts, Mt. Veľká Svišťovka, (WC)	49.20	20.24	1936	13-Aug-14	NS	30
H47	SK, Vysoké Tatry Mts, Mt. Veľká Svišťovka, (WC)	49.20	20.24	1936	28-Aug-14	NS	14

Pop – population code. Country's abbreviation: AT – Austria, BA – Bosnia and Herzegovina, CH – Switzerland, CZ – Czech Republic, FI – Finland, FR – France, NO – Norway, RO – Romania, SK – Slovakia, UA – Ukraine. After short description of the locality, an abbreviation of region is given in parenthesis (AP – Alps, EC – Eastern Carpathians, SC – Southern Carpathians, SU – Sudetes, SV – Scandinavia, WC – Western Carpathians). Number (∑) of capitula used for analysis for random and targeted sampling. NS – not sampled; in the case of random sampling none suitable closed capitulum was found.

Table S2. Summary table for frequency and intensity of seed predation, number of co-occurring vascular plant species in vegetation plots (i.e. species richness, SR), vegetation density of three layers (E – total, E1 – herbs and shrubs, E0 – bryophyte), mean size of capitulum, mean stem height and mean pair-wise Jaccard distance among 5 randomly sampled individuals per population in sexual diploid and asexual triploid populations of *Hieracium alpinum*.

code	***reg-ion***	***∑ capitula fp***	***∑ capitula ip***	***Population means***								
				predation			**vegetation coverage**			**size cap**	**stem height [cm]**	**Ja-ccard**
		ab/pr	**ab/pr**	**fp**	**ip**	**SR**	**E**	**E1**	**E0**			
sexual diploid populations												
C1	EC	66 / 39	16 / 16	0.37	0.50	4.41	0.94	0.63	0.31	82	18.2	ND
C2	EC	104 / 0	NO	0	0	4.07	0.86	0.73	0.14	87	11.7	ND
H57	EC	96 / 6	1 / 3	0.06	0.75	2.48	0.92	0.64	0.28	89	20.5	0.56
H63	EC	43 / 64	14 / 50	0.60	0.78	3.17	0.86	0.79	0.07	77	16.1	0.58
H67	EC	ND	ND	ND	ND	2.85	0.75	0.56	0.19	87	12.0	0.33
H73	SC	41 / 132	54 / 67	0.76	0.55	4.87	0.78	0.59	0.20	63	7.6	0.58
H74	SC	88 / 0	NO	0	0	7.50	0.94	0.92	0.02	52	8.7	0.54
H77	SC	97 / 18	10 / 2	0.16	0.17	5.19	0.94	0.79	0.16	73	13.3	0.58
asexual triploid populations												
H110	AP	32 / 9	5 / 0	0.22	0	6.00	0.71	0.44	0.28	70	11.3	0
H111	AP	7 / 4	2 / 1	0.36	0.33	6.12	0.98	0.75	0.22	78	14.7	0.44
H114	AP	31 / 23	10 / 7	0.43	0.41	5.38	0.91	0.71	0.20	55	11.3	0.44
H20	AP	27 / 7	6 / 1	0.21	0.14	5.24	0.81	0.73	0.08	76	13.6	0.10
H29	AP	NA	NA	NA	NA	6.56	0.83	0.61	0.22	45	7.4	0.23
H3	AP	NA	NA	NA	NA	4.93	0.95	0.81	0.15	68	11.0	0.08
H4	AP	21 / 6	3 / 0	0.22	0	7.40	0.84	0.71	0.13	71	10.1	0.13
H87	AP	73 / 27	23 / 1	0.27	0.04	1.36	0.53	0.50	0.03	84	7.2	0.11
H100	SV	9 / 75	31 / 8	0.89	0.21	3.13	0.90	0.45	0.45	49	11.6	0.07
H101	SV	64 / 52	3 / 2	0.45	0.40	4.34	0.93	0.78	0.15	77	16.3	0.07
H102	SV	2 / 14	1 / 0	0.88	0	5.63	0.87	0.42	0.45	64	12.5	0
H103	SV	49 / 3	NO	0.06	NO	4.20	0.74	0.30	0.44	48	10.1	0
H94	SV	31 / 7	NO	0.18	NO	4.68	0.89	0.65	0.24	68	15.2	0
H95	SV	9 / 79	27 / 20	0.90	0.43	4.46	0.96	0.70	0.26	47	16.9	0.07
H99	SV	25 / 8	1 / 0	0.24	0	4.66	0.79	0.41	0.38	79	14.7	0.07
S3A	SV	6 / 7	NO	0.54	NO	4.69	0.88	0.62	0.27	39	11.2	ND
S3B	SV	NA	NA	NA	NA	5.29	0.83	0.57	0.26	60	13.6	ND
H32	WC	40 / 60	22 / 21	0.60	0.49	3.03	0.92	0.68	0.24	102	13.1	0.07
H33	WC	78 / 23	8 / 7	0.23	0.47	4.13	0.94	0.89	0.05	81	16.0	0.35
H42	WC	66 / 37	4 / 9	0.36	0.69	4.10	0.99	0.77	0.22	116	14.8	0.07

For population locations see Figure 1 and Table S1. Infection rates are presented as: i) frequency of seed predation (fp), total number (∑) of non-infested (ab) *versus* infested (pr) capitula per population, and, as a subset, frequency of capitula with multiple infestation (intensity of seed predation; ip); ii) population means of seed predation (frequency) and multiple seed predation (intensity). Population means of parameters of plant-plant interaction: number of co-occurring vascular plant species (e.g. species richness; SR), vegetation density (E – total, E1 – herbs and dwarf shrubs, E0 – bryophyte). Size of capitulum (size cap) is expressed as mean number of florets per capitulum. Jaccard index mean pair-wise distance based on AFLP markers. Abbreviations: NA – not assessed, NO – no occurrence of multiple infestation.

Figure S1. Vegetation 20 * 20 cm plot surrounding a single sampled plant of *Hieracium alpinum*.

Figure S2. Seed predators of *Hieracium alpinum* flower heads: *Campiglossa guttella* (Rondani, 1870). **A.** Adults emerged from capitula of *H. alpinum*. **B.** Symptoms (traces) of damage caused by larvae of seed predators. **C.** Pupal exuviae of *C. guttella* emerged from a seed of *H. alpinum.* **D.** Multiple infestation by pre-imaginal stages larvae inside a capitulum of *H. alpinum*.

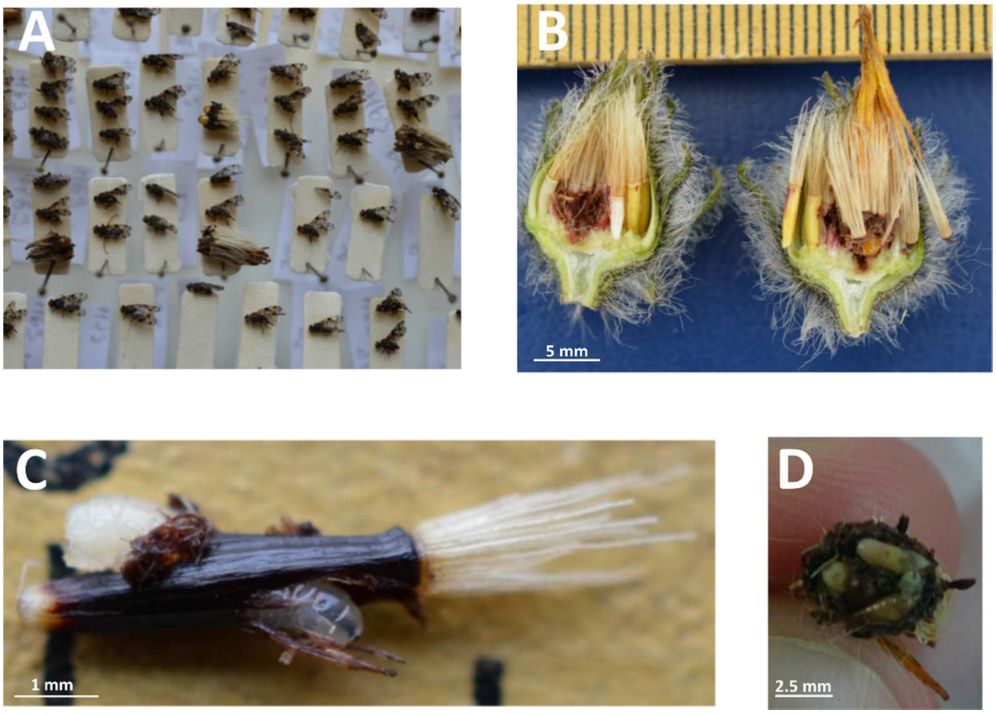


Figure S3. Number of non-infested *versus* infested capitula of *Hieracium alpinum* based on sampling method. Dashed bars indicate not infested, filled bars infested infected capitula.

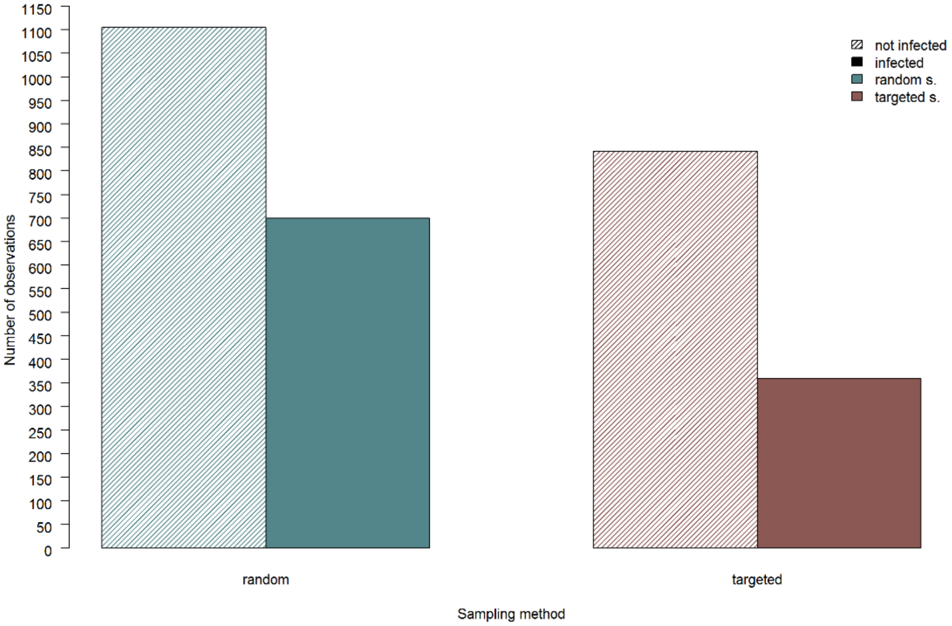


Figure S4. Frequency of seed predation of populations (ploidy level) of *Hieracium alpinum* visited in 2014 and 2015. Dashed bars indicate not infested, filled bars indicate infested capitula.

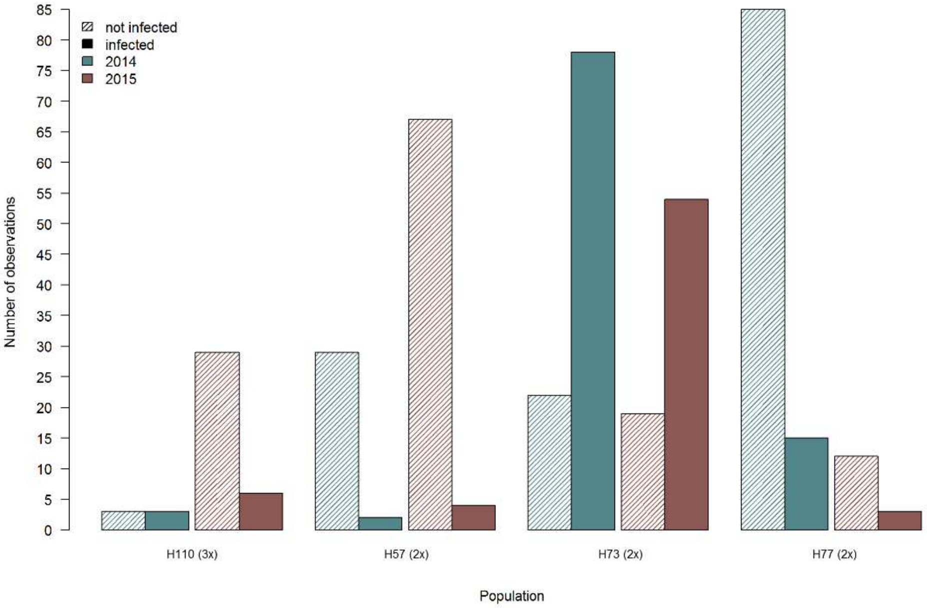


Figure S5. Differences between populations of *Hieracium alpinum* in frequency of seed predation. Bars represent population average of seed predation. Dashed lines refer to the regional average of seed predation the frequency populations occur in.

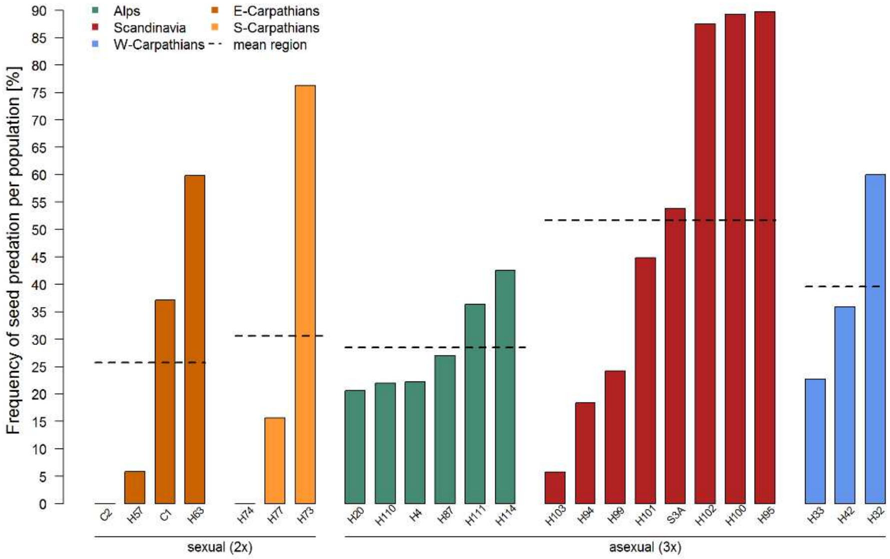


Figure S6. Regional differences in frequency (**A**) and intensity (**B**) of seed predation in sexual diploid and asexual triploid populations of *Hieracium alpinum*. Boxes are based on population means of referring variables. Triangles indicate mean values for referring group. Different letters on top of boxes indicate significant regional differences in intensity of seed predation, obtained from Tukey test.

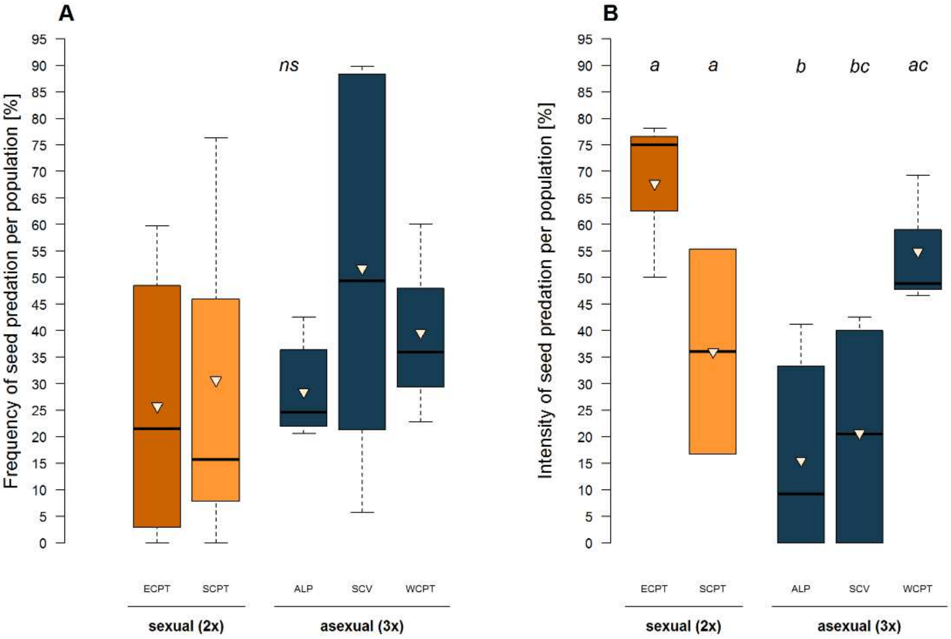


Figure S7. Intensity of seed predation per population of *Hieracium alpinum*. Ratio between multiple infested and non-infested capitula of *H. alpinum.* Filled parts refer to multiple infested, dashed parts to not multiple infested capitula.

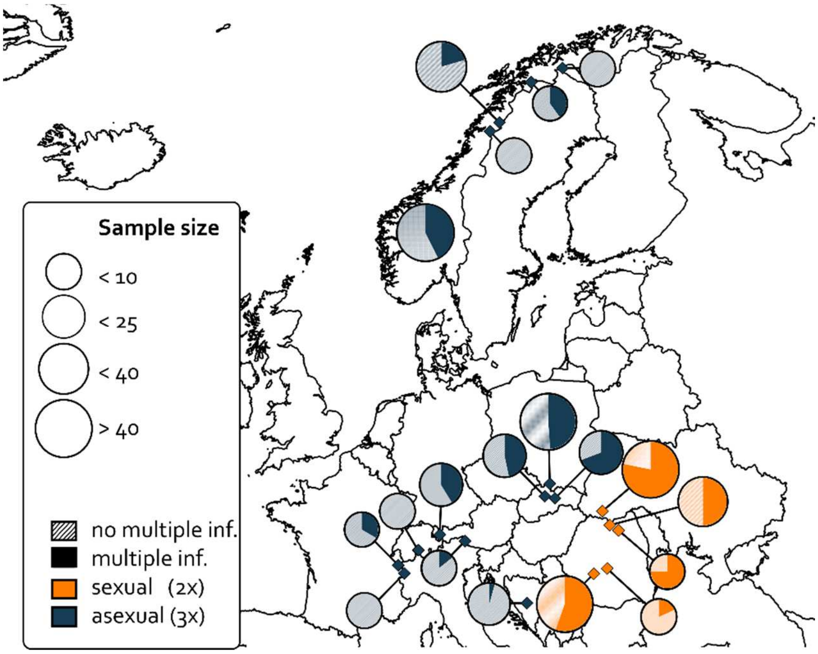


Figure S8. Differences between populations of *Hieracium alpinum* in intensity of seed predation. Bars represent population average of seed predation. Dashed lines refer to the regional average of seed predation frequency populations occur in.

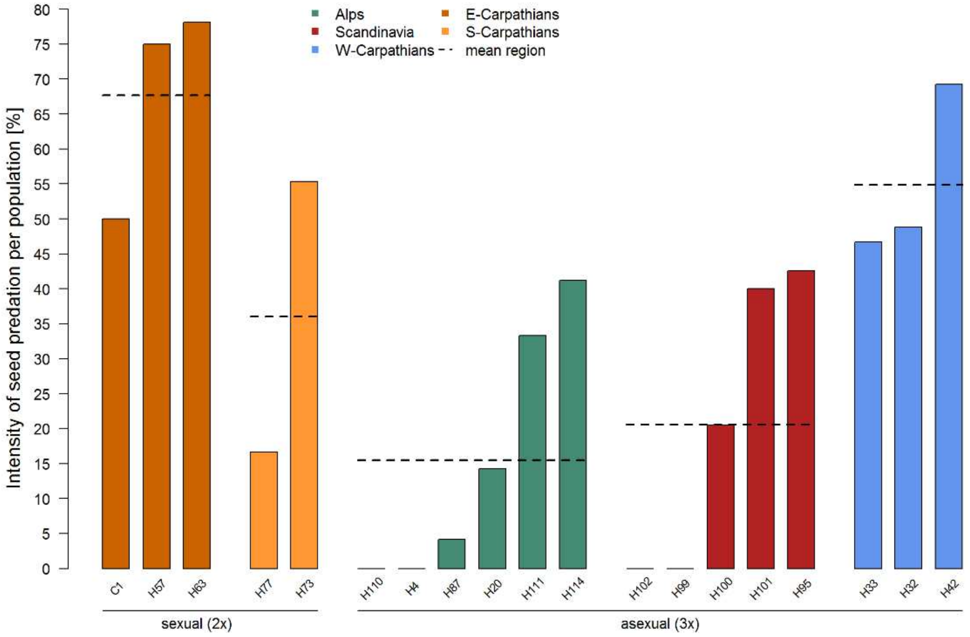
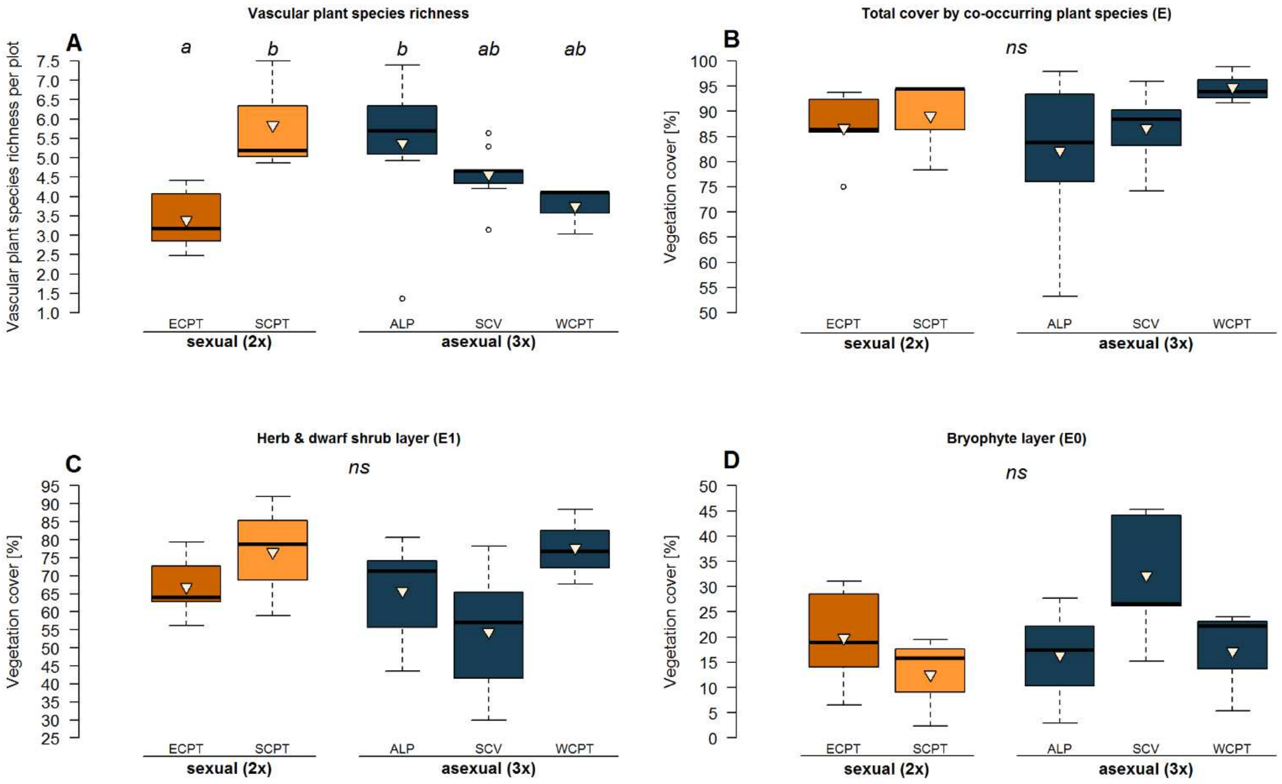


Figure S9. Regional differences in plant-plant interaction parameters in sexual diploid and asexual triploid populations of *Hieracium alpinum*. A. Number of co-occurring vascular plant species (species richness) within a 20 x 20 cm plot surrounding a single sampled plant of *H. alpinum*. B. Percentage of total cover by co-occurring plant species (E). C. Percentage of cover by herb and dwarf shrub layer (E1). D. Percentage of cover by bryophyte layer (E0). Different letters on top of boxes indicate significant regional differences in averaged number of co-occurring vascular plant species, obtained from Tukey test (ns, not significant). Triangles indicate mean values for referring group. Region abbreviations: ALP–Alps, ECPT–Eastern Carpathians, SCPT–Southern Carpathians, SCV–Scandinavia, WCPT–Western Carpathians.



PAPER II

Hartmann, M.; Jandová, K.; Štefánek, M.; Chrtek, J.; Mráz, P.: Effects of latitudinal and elevational gradients exceed the effects of between-cytotype differences in eco-physiological leaf traits in diploid and triploid *Hieracium alpinum*.
resubmitted to *Alpine Botany*

Photos: Habitats of triploid *Hieracium alpinum* populations of H099 (Mt. Hellerfjellet, Norway and H032 (Mt. Babia Hora, Slovakia) (Photo credit: P. Mráz**)**

Effects of latitudinal and elevational gradients exceed the effects of between-cytotype differences in eco-physiological leaf traits in diploid and triploid *Hieracium alpinum*

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Abstract

Polyploidy may affect a species' eco-physiology, which might, in turn, trigger a shift in the distribution of its cytotypes. The arcto-alpine *Hieracium alpinum* (Asteraceae) encompasses two geographically allopatric cytotypes; diploids occurring in the South-Eastern Carpathians and triploids occupying the remaining, much larger part of the species range. We ask whether the natural populations of these two cytotypes, growing under partially different biotic and abiotic conditions, also differ in selected eco-physiological traits. To answer this question, we analyzed specific leaf area, foliar carbon (C) and nitrogen (N) contents and their stable isotope compositions in plants sampled in 27 populations across the species range. Our results did not show any differences in these traits, but foliar N content being significantly higher in diploids. This pattern was mostly driven by the Scandinavian triploid populations exposed to significantly lower amounts of solar radiation and precipitation during the growing season when compared to the continental populations. As a consequence, in addition to lower foliar N content, the Scandinavian populations exhibited also lower foliar C content, but higher C/N ratios than continental populations regardless of their cytotype. Across the species range, foliar N and C contents were positively associated with the amount of precipitation, whilst $\delta^{15}N$ was positively associated with temperature and negatively with the surrounding species richness and vegetation cover. Significantly lower values of $\delta^{13}C$ in Scandinavian populations is likely the effect of increased atmospheric pressure due to the lower elevational position of Scandinavian sites. Reproductive output was positively linked to amounts of foliar nitrogen and $\delta^{15}N$. Our data thus show that (i) the latitudinal driven abiotic and biotic factors affected eco-physiological traits in significantly larger extent than differences in ploidy level and that (ii) continental and Scandinavian populations, though all confined to the alpine belt, considerably differ in their eco-physiology likely reflecting different adaptation strategies.

Keywords: environmental gradients, foliar carbon, foliar nitrogen, geographical parthenogenesis, *Hieracium alpinum*, specific leaf area, stable isotopes, polyploidy

Introduction

Polyploidization is a major speciation mechanism in angiosperms potentially leading to different adaptive potentials of cytotypes (ploidy variants of the same species) (Ramsey and Schemske 1998). The whole genome multiplication may instantaneously trigger various structural and functional changes manifested in altered eco-physiological traits to the surrounding environment (Parisod et al. 2010; Hao et al. 2013). This, in turn, could lead to an allo- or parapatric occurrence of different cytotypes, a frequently reported pattern in vascular plants (Soltis et al. 2007). Accordingly, several studies provided evidences for the different responses of cytotypes to various environmental factors/stresses (Baldwin 1941; Levin 2002; Buggs and Pannell 2007; Maherali et al. 2009; Manzaneda et al. 2012), though there are also other studies which failed to reveal any eco-physiological differentiation between cytotypes (Maherali et al. 2009; Parisod et al. 2010). If cytotypes occupy different distributional ranges, it might be expected that they differ in their eco-physiological traits depending on the local conditions, what surprisingly has been only rarely assessed (Thompson, Husband & Maherali, 2014; but see Maherali et al. 2009).

For plants, the most important eco-physiological processes are those related to photosynthesis and optimal uses of available resources, and thus, promoting long-term fitness (persistence and reproduction). Depending on the abiotic and biotic environment the plant is facing, the plant has to invest either in metabolic (high N content) or in structural components (high C content) what is referred to as productivity-stability-continuum (Reich et al. 1999). Accordingly, the plant's distributional success is determined by its capability to position and/or, if necessary, reposition itself along this continuum in a given heterogeneous abiotic and biotic environment. This renders the C/N ratio to a powerful tool to measure the response of plants to local conditions (Reich et al. 1997; Reich et al. 1999). Using a mathematical model, Mustard et al. (2003) showed that the C/N ratio of plants growing in ruderal habitats was shifted towards N whilst the ratio of plants growing under competitive environments was shifted towards C. However, this pattern could also be influenced by the leaf lifespan (Reich et al. 1999; Wright and Westoby 2002) and nutrient availability (Mustard et al. 2003). Similarly, the C/N ratio is mirrored in the differences in specific leaf area (henceforth SLA) which is defined as the ratio of leaf area to dry mass (Vile et al. 2005). In order to limit the potential transpiration surface, a plant will decrease its leaves' SLA by reducing the leaf area in situations of water-shortage (Craufurd et al. 1999; Casper et al. 2001), which might be even more pronounced in situations of high temperatures (Maron et al. 2007). A further reduction of the proportion of photosynthetic active tissues in the leaf (i.e. lowered foliar N content) will limit the respiration-related water loss (Reich et al. 1999), as the leaves with larger area increase the efficiency of light capture and hence photosynthetic capacity (Field and Mooney 1986; Reich et al. 1999).

The ratio of carbon stable isotopes (henceforth $\delta^{13}C$) is driven by leaf gas exchange conditions during photosynthesis and can be used to infer amounts of available resources like water, CO_2 and radiation and also plants' responses to changes in their availabilities (Farquhar et al. 1989). Under water-shortage, plants reduce the gas exchange and transpiration by closing the stomata. Subsequently, less discrimination against $^{13}CO_2$ molecules in leaf tissues occurs and the values of $\delta^{13}C$ will increase (Körner et al. 2016). Similarly, the lower partial pressure of CO_2 at high elevations causes lower discrimination against ^{13} CO_2 molecules in leaf tissues and increase in $\delta_{13}C$ (elevation effect, e.g. Körner et al 1991, Zhu et al. 2010).

The ratio of nitrogen stable isotopes in plants (henceforth $\delta^{15}N$) can provide valuable information about the nitrogen pools the plant is accessing and the mechanisms involved in their acquisition (Craine et al. 2009). The local abiotic and biotic environment influences the amount of available N and its turnover rates that, in turn, affect foliar $\delta^{15}N$. Rather wet and cold sites are known to be more efficient in conserving and recycling soil N leading to generally lower $\delta^{15}N$ (Craine et al. 2015).

In contrast, rather dry and hot sites enhance more open N cycle with high N losses via volatilization leading to increased levels of foliar N content and $\delta^{15}N$ (Amundson et al. 2003; Craine et al. 2009; Craine et al. 2015). In addition, various nitrogen pools differ in their $\delta^{15}N$ according to the chemical form (organic N > ammonium > nitrates) and soil depth they emerge from (deeper > shallower). Also, the nitrogen acquisition via mycorrhizal symbiosis discriminates more against ^{15}N than N_2-fixation or direct assimilation from soil solution (Michelsen et al. 1996; Hobbie et al. 1999).

To assess the putative differences in eco-physiological traits between cytotypes in their respective ranges we used the arcto-alpine plant *Hieracium alpinum* L. (Asteraceae). The species includes diploid populations reproducing strictly sexually, which are distributed exclusively in the Eastern and Southern Carpathians, thus on the south-easternmost margin of the species range (Fig. 1). The triploid populations, which reproduce strictly asexually (apomictic diplospory; cf. Rosenberg, 1927; Gentscheff & Gustafsson, 1940; Chrtek jun, 1997; Mráz, 2003; Mráz, Chrtek & Šingliarová, 2009), cover the remaining part of the species distributional range (Fig. 1). Such distributional pattern is referred to as geographical parthenogenesis (Vandel 1928; Hörandl 2006). Importantly, a recent molecular study based on ITS, AFLPs, three cpDNA markers, and ca 900 nuclear genes revealed that triploids are of autopolyploid origin (Mráz et al. in prep.). Given this allopatric distribution and much larger distribution of the triploid cytotype, we assume that diploids and triploids respond differently to the local environmental conditions with putatively higher variance in measured traits in the triploid populations due to the larger distributional range covered by triploids. To test these hypotheses, we compared the foliar carbon, nitrogen and their stable isotope compositions, foliar C/N ratio and SLA between both cytotypes sampled across large parts of the species range. Furthermore, we assessed whether the plants' eco-physiological traits follow latitudinal/elevational clines driven by the macroclimatic conditions (derived from the WorldClim2 model) or the local biotic factors, specifically by the density of surrounding vegetation and vascular plant richness. Given the higher metabolic demand of N (and P) of polyploids compared to diploids to build DNA (Lewis Jr 1985; Sterner and Elser 2002; Leitch and Leitch 2008) together with the high nutrient requirements to produce reproductive structures (Sinclair 1975; Mattson Jr 1980; Reekie and Bazzaz 1987), we expect to find some correlations between some important eco-physiological traits and traits important for plants' reproduction and dispersal like flower head size and stem height.

Methods

Sampling

The sampling was performed within the scope of a large research project investigating the causality of geographic parthenogenesis (GP) in *Hieracium alpinum*. In summer of 2014 (end of July and August), we visited 27 populations of *H. alpinum* in its diploid (the Eastern and Southern Carpathians) and triploid ranges (the Alps, the Balkans, Scandinavia, the Sudetes Mts. and the Western Carpathians; Fig. 1). Additional sampling of four new and re-sampling of four populations took place in summer of 2015. At each site, we selected 30 plants at the stage of ripe achenes to measure various traits. The sampling was conducted in two crossed-over linear transects and with a 2 m minimum distance between plants. We took a photo of every plant with the surrounding vegetation within a square of the size of 20 × 20 cm in order to estimate the co-occurring vascular plant species diversity and vegetation density within the plot (Hartmann et al. 2017). In order to assess the specific leaf area (SLA) and to quantify the foliar C, N contents and their stable isotope compositions ($\delta^{13}C$ and $\delta^{15}N$), we selected 10 out of 30 plants in each population. Three well-developed, healthy leaves per selected plant were sampled from the central part of the rosette and immediately herbarized in the field to get flat samples suitable for measurements of SLA. The dried and herbarized leaves

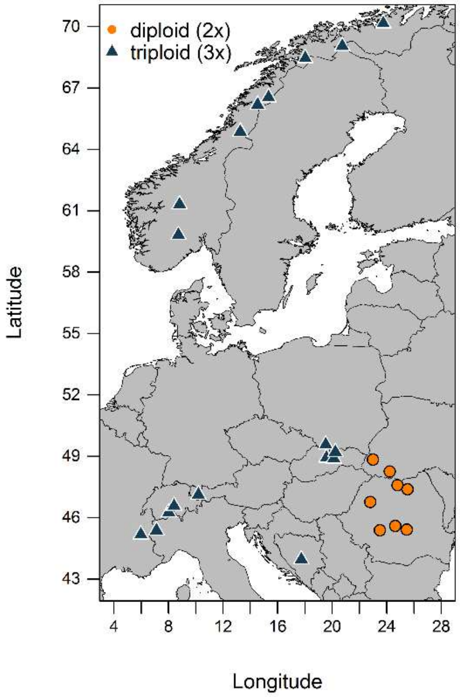

Fig. 1: Locations of *Hieracium alpinum* sampling sites. Symbols correspond to the ploidy level: 2x — diploid, 3x — triploid level

were scanned using a flatbed scanner and transformed into binary images from which the leaf area was calculated using ImageJ software (Schneider et al. 2012). Herbarized leaves were dried at 60°C for 48h before weighing. The SLA was calculated as the ratio of leaf area to dry mass (Vile et al. 2005) and mean value of three leaves was calculated for each individual plant.

Determination of foliar C and N content and their stable isotope composition

For isotope-ratio mass spectrometry (IRMS), all three leaves per plant were homogenized as a bulk sample using a ball mill (MM 400, Retsch, Germany) and weighted into tin capsules. The total C and N content and their isotopic ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were determined using an elemental analyzer Flash 2000 with TCD detector coupled to Conflo IV and mass spectrometer Delta V Advantage (Thermo Scientific, Bremen, Germany) in the Center for Stable Isotope Research of the Charles University, Prague. The isotope ratios were expressed as δ values (in per mill, ‰) versus a standard (Vienna Pee Dee Belemnite and atmospheric N_2 for C and N, respectively) using a formula: $\delta = 1000 \times (R_{sample} - R_{standard}) / R_{standard}$, where R is the relative abundance of the heavy to that of the light isotope. The analytical precision of measurements was estimated using 22 replicates of the internal standard (homogenized *Betula pendula* leaves). The analytical precision (standard deviation) was within ± 0.1 ‰ for both isotopes, within ± 0.04 % for foliar C content and within 0.013% for foliar N content.

Statistical analyses

Firstly, we tested for putative associations among the measured traits (SLA, foliar C and N content and their stable isotope composition) on plant level using Pearson correlation tests. In order to assess the effect of ploidy level (diploid *versus* triploid; a fixed effect) on i) the foliar C and N content, ii) foliar $\delta^{15}N$, iii) the C/N ratio, iv) foliar $\delta^{13}C$, and v) SLA, we used linear mixed effect models (LMM) with the identity link function for normally distributed data. Assuming more similar biotic and abiotic environments among geographically related populations and based upon the spatial orientation of the mountain ranges (Mráz et al. in prep.), each population was attributed to one of the five mountain ranges (regions): the Alps, the Eastern Carpathians, the Southern Carpathians, Scandinavia, and the Western Carpathians. Population was used in the models as a random term (Table S1). All analyses were performed within the R environment (R Core Team 2014) using *lmer* functions implemented in the lme4 package (Bates et al. 2014) and statistical significance of terms was assessed using likelihood ratio test (LRT) comparing a fully fitted model to the model from which the tested term was removed. To account for different levels of variances between ploidies and among populations (confirmed by Levene tests), we used *varIdent* function implemented in the nlme package (Pinheiro et al. 2017).

Given the huge latitudinal gradient covered by our sampling, we also tested for latitudinal clines of leaf variables. The tests were conducted on plant level with the leaf variables (fixed effect) and with the populations' latitudinal position as explanatory variable in the same manner as above but with population nested within region as random effect. Moreover, we used populations' elevational position as explanatory variable to test whether the decreased partial pressure of CO_2 with increasing elevation will affect $\delta^{13}C$.

In order to assess the effect of the abiotic factors on the leaf traits, we performed LMM tests using the plants' leaf traits (explanatory variable) in the same manner as above but with the selected populations' climatic variables as fixed effect. Assuming the highest impact of climate on plant eco-physiology during the growing season (May-September for alpine plants), we calculated mean temperature, cumulative amount of precipitation and cumulative amount of solar radiation values for this period of a year (henceforth TDUGS, PDUGS, SDUGS). The calculations were based on data available from the WorldClim 2 model within 30sec (~1 km^{-2}) resolution (Fick and Hijmans 2017). In our study, we consider SDUGS as a proxy for the energy an environment is receiving, allowing us to get an insight into its productivity. We performed two principal component analyses implemented in the ade4 package (Dray and Dufour 2007) to visualize the relationship among diploid and triploid populations based on (i) three macroclimatic variables, i.e. TDUGS, PDGUS, SDUGS; and (ii) all eco-physiological leaf traits but the C/N ratio, which was strongly correlated with the foliar N content (see below).

We further tested for the putative impact of the biotic environment on the leaf traits. To this aim we performed LMM tests on individual plant level with the foliar N and C content, the C/N ratio and $\delta^{15}N$ as response variable, and the number of co-occurring vascular plant species, the total cover, the cover by bryophytes and lichens and the cover by herbs as explanatory variable. Population nested within region was used as a random effect.

Tests of covariance between some important eco-physiological leaf traits and traits important for dispersal and reproduction, i.e. stem height (the height where the single inflorescence – flower head or capitulum – with seeds is situated) and inflorescence size (expressed as number of florets per capitulum) were conducted using non-parametric correlation tests (Spearman).

Results

Associations among the eco-physiological leaf traits

The tests of putative associations amongst the measured traits revealed a significant negative correlation between SLA and the $\delta^{13}C$ ($\rho = -0.33$, $P < 0.001$), but a positive between SLA and foliar N content ($\rho = 0.25$, $P < 0.001$; Fig. S1). The foliar C and N contents were positively associated with $\delta_{13}C$ ($\rho = 0.31$, $P < 0.001$; $\rho = 0.28$, $P < 0.001$, respectively) and $\delta^{15}N$ ($\rho = 0.26$, $P < 0.01$; $\rho = 0.21$, $P < 0.001$, respectively). The positive association between $\delta^{13}C$ and foliar N content across all sampled populations was solely driven by Scandinavian triploid populations (Pearson parametric test in: 2x populations: $\rho = 0.105$, $P > 0.05$; 3x: $\rho = 0.407$, $P < 0.001$; continental European 3x populations: $\rho = 0.139$, $P > 0.05$; Scandinavian 3x populations: $\rho = 0.345$, $P < 0.01$).

Differences in eco-physiological leaf traits and their variances between the cytotypes

In general, diploid plants were characterized by higher values of all measured leaf traits but the C/N ratio (Fig 2; Table S1). However, the tests rejected any significant differences between the cytotypes but a significant higher foliar N content and, derived, significantly smaller C/N ratio in diploids when compared to triploids (Table 1, Fig 2). When studied more closely, we found that these differences were caused by the triploid populations from Scandinavia having the lowest N values and highest C/N values (Table S1, Fig 2). Consequently, when Scandinavian populations were excluded from the analyses (Table 1), we did not find any significant differences in foliar N content and C/N ratio between the cytotypes suggesting an important impact of the plants from Scandinavia on the overall results (Table 1, Fig 2). The models controlling for heteroscedasticity confirmed the previous results.

Table 1. Results of likelihood ratio tests (χ^2 values in the first column and p values in the second column) of linear mixed effect models (LMMs) examining the differences in leaf eco-physiological traits between diploid and triploid cytotypes of *Hieracium alpinum*. Population was included in the models as a random factor. Two different datasets were used with (full model) and without Scandinavian populations (without Scandinavia; see Material and Methods).

	Leaf traits														
Fixed and random effects/ response variables	**foliar C content**		**foliar N content**			**C/N ratio**			**$\delta^{15}N$**		**$\delta^{13}C$**			**SLA**	
full model$^{\#}$															
ploidy	2.35	*NS*	**9.56**	**	↓	**9.6**	**	↑	0.61	*NS*	0.001	*NS*		1.58	*NS*
without Scandinavia															
ploidy	0.05	*NS*	3.05	0.08	↓	3.17	0.1	↑	0.69	*NS*	**7.01**	**	↓	1.87	*NS*

The direction of arrows indicates whether asexual triploid populations reached lower (↓) or higher (↑) values when compared to sexual diploid populations.
Abbreviations: *NS* – not significant; NT – not tested; ** $P < 0.01$; for marginally significant differences exact *P*-values are given.

We found significantly higher variation in the foliar C content (F = 5.7, $P < 0.05$), the C/N ratio (F = 14.2, $P < 0.001$), the $\delta^{13}C$ (F = 11.9, $P < 0.001$), and significantly lower variation in SLA (F = 5.8, $P < 0.05$) in triploids compared to diploids (Fig 2).

The variance partitioning analyses (Table 2; Figs S2, S3) showed that more of the observed variation in the measured traits was explained by the abiotic environment when compared to the biotic environment (averaged variation explained by the abiotic environment was 23.9%, whilst that by the biotic environment was 7.3%; Table 2). This applied to all traits but $\delta^{15}N$, in which the averaged variation explained by the abiotic environment (15.3%) was lower than the proportion of explained variation due to the biotic environment (20.4%; Table 2, Figs S2, S3). Across all traits, PDUGS, as single factor or shared with other factors, was the main driver of the observed variation (Table 2,Fig S3). However, for $\delta^{13}C$ the most variation could be explained by the populations' elevational position (as a single factor or shared with others; Table 2,Fig S2). Considering the biotic environment, vascular plant species richness alone or in interaction with total vegetation cover explained most of the observed variation in δ^{15}N, whilst for δ^{13}C the cover of either vascular or non-vascular plant species or the total cover explained most of the observed variation (Table 2, Fig S3).

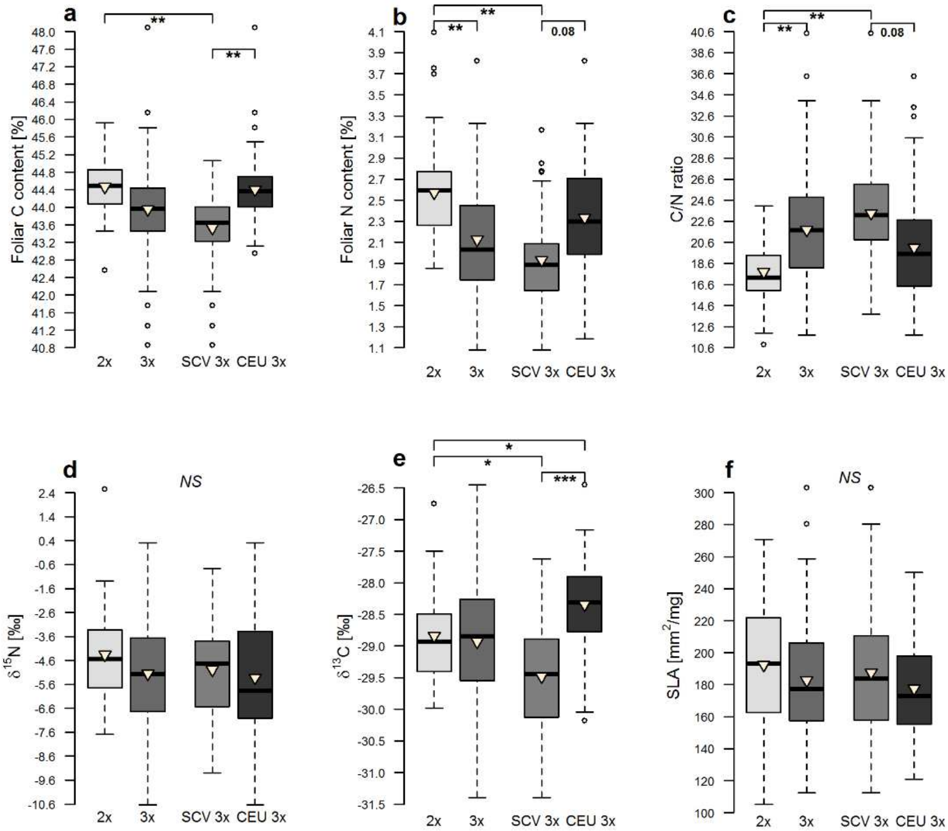

Fig.2: Boxplots of eco-physiological leaf traits in diploid (2x) and triploid (3x) populations of *Hieracium alpinum* and Scandinavian triploid (SCV 3x) and continental European triploid (CEU 3x) populations. a, Foliar carbon content. b, Foliar nitrogen content. c, Foliar carbon to nitrogen ratio. d, Foliar δ^{15}N. e, Foliar δ^{13}C. f, Specific leaf area (SLA). Triangles indicate mean values. Only statistically significant (or marginally significant) differences are displayed. *NS*, not significant ($P > 0.1$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; for marginally significant differences, exact *P*-values are given.

Latitudinal and elevational clines and associations between the leaf eco-physiological traits and abiotic and biotic factors

When searching for putative associations between the measured variables and the populations' latitudinal position, we found significantly negative patterns in foliar C and N contents and δ^{13}C, and significantly positive latitudinal clines in the C/N ratio (Table 3, Fig S4). In contrast, no statistically significant association between latitude and δ^{15}N and SLA were found (Figs S4, S5). The δ^{13}C values significantly increased with increasing elevation across all populations ($\chi^2 = 12.11$, $P < 0.001$; Fig 3), a trend driven by triploid populations, as no such association was found within the diploids' range (2x: $\chi^2 = 2.95$, $P = 0.086$; 3x: $\chi^2 = 8.58$, $P < 0.01$.)

Table 2. Results of the variance partitioning analyses estimating the explained proportions of the observed variation in the eco-physiological leaf traits of diploid and triploid *Hieracium alpinum* plants using the plants' cytotype and abiotic and biotic environment. Exclusively for δ^{13}C, the populations' elevational position was used. The adjusted R² of the referring partitions are given (see also Figs S3, S4). The adjusted R² depend on the number of observations and number of predictors in the analysis and given the nature of how it is calculated, adjusted R² can become negative. Abbreviations: TDUGS - Mean Temperature during growing season [°C], PDUGS - Amount of precipitation during growing season [mm], SDUGS - Amount of solar radiation during growing season [MJ m^{-2} day^{-1}], ELEV - Populations' elevational position, Richness – number of co-occurring vascular plant species (species richness); Cover E1 – herb and dwarf shrub layer cover, Cover E0 – bryophyte and lichen layer cover; NT — not tested.

	Leaf traits					
	δ^{15}N	**$\delta^{13}C^{\#}$**	**foliar N content [%]**	**foliar C content [%]**	**C/N ratio**	**SLA**
abiotic environment						
Residuals	0.847	0.589	0.758	0.659	0.753	0.956
Total	0.153	0.411	0.242	0.341	0.247	0.044
ELEV	NT	0.353	NT	NT	NT	NT
TDUGS	0.108	0.066	0.037	0.001	0.040	0.025
PDUGS	0.014	0.138	0.237	0.347	0.240	0.002
SDUGS	0.041	0.181	0.030	0.085	0.027	0.015
TDUGS+PDUGS	0.110	0.251	0.247	0.344	0.251	0.023
TDUGS+SDUGS	0.124	0.207	0.090	0.103	0.090	0.031
PDUGS+SDUGS	0.110	0.218	0.235	0.344	0.238	0.040
TDUGS+PDUGS+SDUGS	0.153	0.280	0.242	0.341	0.247	0.044
ELEV+TDUGS	NT	0.353	NT	NT	NT	NT
ELEV+PDUGS	NT	0.351	NT	NT	NT	NT
ELEV+SDUGS	NT	0.417	NT	NT	NT	NT
ELEV+TDUGS+PDUGS	NT	0.355	NT	NT	NT	NT
ELEV+TDUGS+SDUGS	NT	0.414	NT	NT	NT	NT
ELEV+TDUGS+PDUGS+SDUGS	NT	0.411	NT	NT	NT	NT
biotic environment						
Residuals	0.796	0.999	0.932	0.986	0.936	0.912
Total	0.204	0.001	0.068	0.014	0.064	0.088
Richness	0.166	-0.006	0.007	-0.005	-0.001	0.016
E1 cover	0.025	0.007	0.045	0.000	0.052	0.047
E0 cover	-0.005	0.012	0.048	0.021	0.052	0.057
Richness+E1 cover	0.168	0.002	0.067	-0.004	0.063	0.084
Richness+E0 cover	0.166	0.007	0.060	0.016	0.055	0.082
E1 cover+E0 cover	0.078	0.007	0.048	0.019	0.055	0.056
Richness+E1 cover+E0 cover	0.204	0.001	0.068	0.014	0.064	0.088

Table 3. Results of likelihood ratio tests (χ^2 values in the first column and p values in the second column) examining relationships between latitudinal positions of *Hieracium alpinum* populations and the leaf eco-physiological traits on plant level. Linear (LMM) models, with population nested within a region as random effect, were applied on three different datasets separately: complete data set (containing both diploid and triploid populations), and diploid, and triploid data sets, respectively.

	Dataset							
leaf traits	complete			diploid		triploid		
foliar N content	3.50	0.06	↓	0.82	*NS*	1.85	*NS*	
foliar C content	**4.63**	*****	↓	1.77	*NS*	2.86	0.09	↓
C/N ratio	**3.97**	*****	↑	0.53	*NS*	2.04	*NS*	
$\delta^{15}N$	0.20	*NS*		0.44	*NS*	0.53	*NS*	
$\delta^{13}C$	**7.14**	******	↓	1.88	*NS*	**8.67**	******	↓
SLA	0.02	*NS*		0.59	*NS*	0.97	*NS*	

The directions of statistically significant associations indicate whether the referring eco-physiological leaf trait decreases (↓) or increases (↑) with increasing latitude. Abbreviations: NS – not significant; * $P < 0.05$, ** $P < 0.01$; for marginally significant differences exact P-values are given.

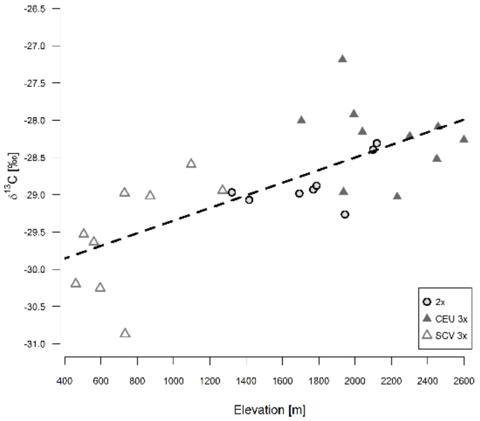

Fig 3: Association between population-averaged foliar $\delta^{13}C$ of diploid (2x) continental European triploid (CEU 3x) and Scandinavian triploid (SCV 3x) populations of *Hieracium alpinum* and the referring elevational position.

The populations from the diploid range were exposed to the similar precipitation and irradiance conditions, but to significantly higher temperatures when compared to the populations from the triploid range (ANOVA tests: PDUGS: F = 1.22, $P > 0.05$; SDUGS: F = 2.58, $P > 0.05$; TDUGS: F = 5.7, $P < 0.05$; Table S1; Figs. 4, S6). The latter pattern was driven by continental triploid populations, which were exposed to significantly lower TDUGS. Moreover, when comparing populations from Scandinavia with those from continental Europe (both diploid and triploid populations together), the former were exposed to significantly lower PDUGS and SDUGS (PDGUS: F = 14.57, $P < 0.001$; SDGUS: F = 21, $P < 0.001$), but exhibited similar values of TDUGS (F = 1.3, $P > 0.05$; Table S1; Figs. 4, S6). The obvious differences in macroclimatic conditions between

Scandinavian and continental populations were revealed also by principle component analysis (Fig 4A). Interestingly, such profound differentiation was only slightly mirrored in the multivariate eco-physiological space showing some differentiation of the Scandinavian populations due to lower foliar C and foliar N contents (Fig 4B).

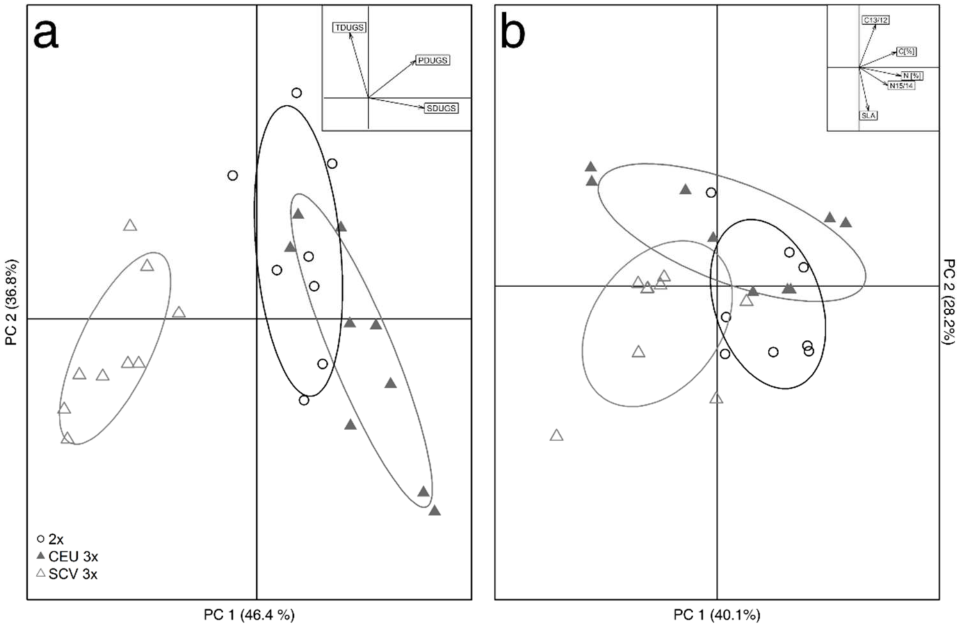

Fig 4: Principle component analysis (PCA) of the local abiotic environment (A) and eco-physiological leaf traits (B) in diploid (2x) and Scandinavian triploid (SCV 3x) and continental European triploid (CEU 3x) populations of *Hieracium alpinum*. Abiotic environment abbreviations: TDUGS – Mean temperature during growing season (May – September); PDUGS – Amount of precipitation during growing season (May – September); SDUGS – amount of solar radiation during the growing season (May – September). Leaf trait abbreviations: CN_rat — Foliar carbon to nitrogen ratio; SLA — specific leaf area; N15/14 — Foliar δ^{15}N; N [%] — Foliar N content; C [%] — Foliar C content; C13/12 — Foliar δ^{13}C.

Across all populations, the amount of foliar C and N content was significantly associated with higher PDUGS, which was mostly driven by the triploid populations (Table 4). Moreover, there was a marginally significant increase in SLA and a significant increase in the values of δ^{15}N with increasing TDUGS across all visited populations, a trend, which was likely caused by the triploid populations (Table 4, Fig 5). Considering diploid populations only, we found a significant negative correlation between δ^{13}C and TDUGS (Table 4, Fig 5).

When testing for possible associations between the leaf traits and the biotic environment, we found that higher vascular species richness, total vegetation cover and the proportion of herbaceous plants in the 20×20 cm plots lead to more negative $\delta^{15}N$ values. These patterns were mainly due to more different values recorded in the diploid populations (Table 4). Moreover, the foliar N content decreased, and the C/N ratio increased with increasing proportion of bryophytes and lichens in the vegetation plots. Decreasing foliar C content was associated with a higher total cover by co-occurring vegetation, a pattern, which was driven by diploid populations (Table 4).

Table 4. Results of likelihood ratio tests (χ^2 values in the first column and p values in the second column) examining relationships between abiotic (mean temperature [TDUGS] and cumulative amount of precipitation [PDUGS] derived from the WorldClim2 model during growing season – from May to September) and biotic (vascular plant species richness and density of surrounding vegetation) variables in *Hieracium alpinum* populations and the eco-physiological leaf traits. Linear (LMM) models, with population nested in region as random effects, were applied on three different datasets separately: complete data set (containing both diploid and triploid populations), and diploid, and triploid data sets, respectively.

		Abiotic factors						***Biotic factors***											
	Dataset	**TDUGS**			**PDUGS**			**Richness**			**E cover**			**E1 cover**			**E0 cover**		
foliar N content	complete	2.14	*NS*		**4.33**	*	↑	0.19	*NS*		0.16	*NS*		2.29	*NS*		**5.36**	*	↓
	diploid	0.94	*NS*		0.50	*NS*		3.96	*	↓	0.29	*NS*		0.39	*NS*		2.29	*NS*	
	triploid	1.27	*NS*		**11.50**	***	↑	0.22	*NS*		0.08	*NS*		0.73	*NS*		1.73	*NS*	
foliar C content	complete	0.03	*NS*		**16.23**	***	↑	0.25	*NS*		**5.70**	*	↓	0.84	*NS*		1.12	*NS*	
	diploid	0.56	*NS*		**9.19**	**	↑	2.84	0.092	↓	**4.27**	*	↓	1.11	*NS*		0.24	*NS*	
	triploid	0.08	*NS*		**10.38**	**	↑	1.51	*NS*		3.04	0.081		0.27	*NS*		0.95	*NS*	
C/N ratio	complete	2.33	*NS*		**5.50**	*	↓	0.08	*NS*		0.05	*NS*		3.11	0.078	↓	**6.05**	*	↑
	diploid	0.68	*NS*		0.85	*NS*		3.35	0.067	↑	0.21	*NS*		0.41	*NS*		2.34	*NS*	
	triploid	1.34	*NS*		**11.51**	***	↓	0.21	*NS*		0.00	*NS*		1.35	*NS*		2.16	*NS*	
$\delta^{15}N$	complete	**4.16**	*	↑	1.05	*NS*		**9.34**	**	↓	**6.50**	*	↓	0.52	*NS*		1.97	*NS*	
	diploid	0.93	*NS*		1.09	*NS*		**12.22**	***	↓	**12.14**	***	↓	**4.11**	*	↓	0.22	*NS*	
	triploid	2.65	*NS*		0.37	*NS*		2.53	*NS*		0.78	*NS*		0.23	*NS*		1.96	*NS*	
$\delta^{13}C$	complete	0.80	*NS*		0.00	*NS*		0.91	*NS*		1.17	*NS*		0.45	*NS*		0.03	*NS*	
	diploid	**5.33**	*	↓	0.87	*NS*		0.06	*NS*		0.50	*NS*		0.01	*NS*		0.26	*NS*	
	triploid	0.06	*NS*		0.05	*NS*		0.53	*NS*		0.77	*NS*		0.39	*NS*		0.002	*NS*	
SLA	complete	2.77	0.096	↑	0.44	*NS*		NT			NT			NT			NT		
	diploid	0.17	*NS*		0.30	*NS*	↑	NT			NT			NT			NT		
	triploid	1.83	*NS*		0.03	*NS*		NT			NT			NT			NT		

The directions of statistically significant associations between leaf traits and environmental variables are indicated with arrows. Abbreviations: richness – number of co-occurring vascular plant species (species richness); E cover – total vegetation cover, E1 – herb and dwarf shrub layer cover, E0 – bryophyte and lichen layer cover; NS – not significant $P > 0.1$); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; for marginally significant differences exact *P*-values are given.

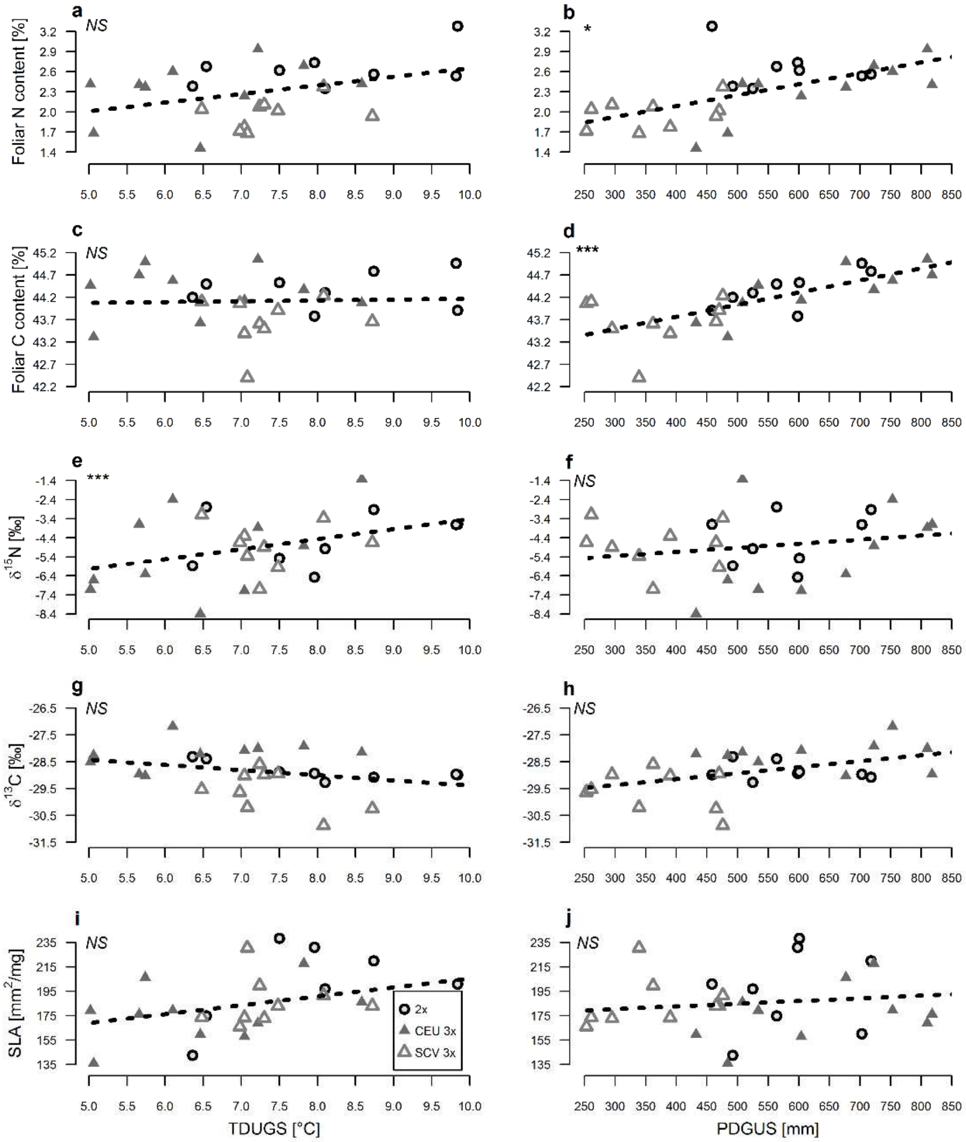

Fig 5: Associations between population-averaged values of eco-physiological leaf traits of diploid (2x), continental European triploid (CEU 3x) and Scandinavian triploid (SCV 3x) populations of *Hieracium alpinum* and the local abiotic environment. Overall associations are indicated by dashed lines. Eco-physiological leaf traits: Foliar N content (a, b); Foliar C content (c, d); foliar δ^{15}N (e, f); foliar δ^{13}C (g, h); SLA, specific leaf area (i, j). Abiotic environment abbreviations: TDUGS – mean temperature during the growing season (May – September). PDUGS – amount of precipitation during the growing season (May – September).

Relationship between leaf eco-physiological traits and traits important for dispersal and reproduction

The capitulum size and foliar N content and δ^{15}N were positively and significantly associated across all populations (Table 5, Fig S7). These positive associations were mostly due to triploid plants and were even more pronounced when Scandinavian plants were excluded as no such associations were found in the latter. The height of diploids' stems marginally and those of triploids, when Scandinavian plants were excluded, significantly increased with foliar N content. Furthermore, the exclusion of Scandinavian plants from the analysis resulted in significant positive associations between the stem height and foliar N content and δ^{15}N, as well as between the capitulum size and these eco-physiological leaf traits.

Table 5. Results of non-parametric correlation tests (Spearman) between eco-physiological leaf traits and traits important for dispersal (Height of inflorescence — Stem height) and reproduction (Size of the capitulum — Cap. Size) of *Hieracium alpinum* across all sampled plants and in diploid (2x), Scandinavian triploid (SCV 3x) and continental European triploid (CEU 3x) plants.

		Dataset									
Leaf trait	Plant trait	complete		2x		3x		CEU 3x		SCV 3x	
δ^{15}N	Stem height	0.025	*NS*	0.032	*NS*	0.051	*NS*	**0.278**	*	-0.233	0.052
	Cap. Size	**0.196**	**	-0.008	*NS*	**0.233**	**	**0.456**	***	0.025	*NS*
foliar N [%]	Stem height	0.065	*NS*	0.243	0.06	0.068	*NS*	**0.261**	*	0.044	*NS*
	Cap. Size	**0.217**	**	0.198	*NS*	**0.180**	*	**0.252**	*	-0.105	*NS*

Abbreviations: *NS* – not significant *P* > 0.1); **P* < 0.05; ***P* < 0.01; ****P* < 0.001; for marginally significant differences exact *P*-values are given.

Discussion

Despite the allopatric distribution of *H. alpinum*'s cytotypes, our data showed that its cytotypes did not differ in their eco-physiological leaf traits, except for the significantly higher foliar N content in the diploids compared to the triploids. Furthermore, triploid populations varied to a greater extent in their eco-physiological traits than diploid populations, likely because triploids occupy a much larger distributional range, characterized by a greater variation in the local abiotic and biotic environments. The most important trends we found were caused by the populations from Scandinavia growing under considerably different environmental conditions when compared to the populations from continental Europe, irrespective of their ploidy level.

Associations among the eco-physiological leaf traits

We found statistically significant positive associations among several eco-physiological leaf traits of *H. alpinum*, but the association between SLA and $\delta^{13}C$, which was negative. Since plants aim to optimize the light capture and, possibly, limit the transpiration related water loss under the given abiotic and biotic conditions, all eco-physiological leaf traits are highly interlinked. Alterations of the leaf thickness and/or leaf size will, therefore, have an impact on the SLA, transpiration surface and the amount of light passing through the leaf on one hand, but also on the foliar N content. Overall, this influences the photosynthetic capacity (Körner et al. 1996; Reich et al. 1999; Evans and Poorter 2001; Wright and Westoby 2002). The positive association between SLA and foliar N content is thought to be linked to an increased photosynthetic capacity (Wright and Westoby 2002). Similarly, in our study, we found a significant positive association between SLA and foliar N content across all sampled populations (Fig S1). Interestingly, considering the Scandinavian populations only, there was no such association. This pattern will be discussed in detail in the last section of the discussion. The found associations between the SLA and $\delta^{13}C$ are likely to be confounded by the plants' elevational position (see below). The found positive association between the foliar N content and δ_{15}N corroborates the global pattern showing higher δ^{15}N values in plants with higher N (Craine et al. 2009; and references therein) and demonstrates how a plant's δ^{15}N can serve as a good proxy for its N status, as suggested by Körner et al. (2016). Given the strong latitudinal cline in foliar N content and elevational cline in δ^{13}C, the positive association between these two eco-physiological traits in Scandinavian populations, describes the overall joint effect of elevation and latitude of our study. The more northerly situated plants are characterized by lowered foliar N contents and, simultaneously, discriminated more against the heavier $^{13}CO_2$ molecules, due to their lowered elevational position.

Differences in eco-physiological leaf traits and their variances between the cytotypes

Diploid *H. alpinum* plants contained in average 20% more N than triploid plants, mainly because triploid plants from Scandinavia had substantially less foliar N than the plants from continental Europe. This is likely because the Scandinavian sites receive much less energy due to their high latitudinal position which, in turn, causes a considerable decrease in net primary production (Sitch et al. 2003) leading to lower amount of available nutrients in the soil, including nitrogen (Bales 2015). Consequently, this could have a negative impact on the plants' reproductive output (see below).

Diploid plants were characterized by almost 20% higher δ^{15}N values than triploid plants, i.e. the former were enriched in ^{15}N isotope compared to the latter. These results corroborate previous findings (e.g. Craine et al. 2009) showing higher δ^{15}N values in plants with higher N content. The foliar N isotopic composition reflects that of the soil N pools accessed by the plant and the mechanism of N acquisition (Craine et al. 2009; Körner et al. 2016). We suppose that the triploid populations from sites with less available N depend increasingly on mycorrhizal symbioses which are known to cause a decrease of foliar δ^{15}N (Hobbie et al. 1999; Craine et al. 2009). However, our analyses rejected any significant differences between the cytotypes in the $\delta^{15}N$ values due to the considerable high intra- and inter-population variation, which was, however, not caused by the high sample size of our study ($\rho = 0.328$, $P = 0.09$) as suggested by Craine et al. (2015).

The cytotypes of *H. alpinum* did not differ significantly in $\delta^{13}C$ due to the huge variation within the triploids because of greater elevational variation of triploid sites. Accordingly, most of the observed variation in this trait could be explained by the populations' elevational position (Körner et al. 1991; Körner et al. 2016).

The diploids did not differ from the triploids in the SLA measured under natural conditions. Similarly, a subsequently performed greenhouse experiment, in which plants of both cytotypes were cultivated under standardized conditions, did not reveal any differentiation between the cytotypes in SLA (Hartmann et al. in prep.). Therefore, this might suggest that polyploidization had no effect on this trait in *H. alpinum*. While some studies found that polyploids have reduced SLA when compared to diploids (Brock and Galen 2005; Hull-Sanders et al. 2009; Mráz et al. 2011), the other found no differences (Thébault et al. 2011). Such inconsistency might stem from the fact that SLA, as a highly adaptive trait, might have evolved differently under different environments. Reducing the SLA in one environment might not be advantageous in a different environment (e.g. Evans and Poorter 2001).

Though diploid plants contained only 1.2% more C than triploid plants, we found a significant negative latitudinal cline in this trait, which was driven by Scandinavian plants containing, in average, 2% less amount of foliar C when compared to continental plants. Interestingly, other studies reported less variation in this trait (Poorter et al. 1995; Poorter et al. 1997; Körner et al. 2016) and no C limitation in alpine plants (Inauen et al. 2012). The small, but highly significant reduction in foliar C content in Scandinavian populations could be due to the overall lower level of metabolism / photosynthesis due to putative interactions between (i) the lower amount of soil nutrients (Bates 2015), which was mirrored in the significantly lowered foliar N contents of Scandinavian plants, (ii) lower energetic input (solar iradiance) in high latitudes (Iqbal 1983; Sitch et al. 2003) and (iii) lower amount of precipitation during growing season (Fig S6).

Latitudinal and elevational clines and associations between the leaf eco-physiological traits and abiotic and biotic factors

The temperature and precipitation directly influence all physiological processes (Lloyd and Taylor 1994; Hobbie et al. 2000; Welker et al. 2003; Reich and Oleksyn 2004; Körner et al. 2016). Whereby the environment a plant is facing predictably varies across latitudinal gradients and determines a plant's eco-physiological response (Maron et al. 2007). In our study, the PDUGS population values were significantly associated with the populations' latitudinal position, whilst the values of TDUGS were not (Spearman non-parametric tests for TDGUS: $\rho = 0.14$, $P > 0.05$; PDUGS: $\rho = -0.42$, $P < 0.05$). Consequently, with exception of $\delta^{13}C$, the observed latitudinal clines in our study were driven mostly by PDGUS.

In our study, whilst the foliar N content was positively associated with PDUGS, which was driven by continental European triploid populations having high foliar N content and receiving high amounts of precipitation, the values of $\delta^{15}N$ were positively associated with TDUGS. The variation in this trait was explained by the abiotic environment to a greater extent when compared to the biotic environment. The values of $\delta^{15}N$ were positively associated with TDUGS, which agrees with the results of Amundson et al. (2003), who showed a positive correlation between the plant's $\delta^{15}N$ and mean annual temperature studied at 106 sites. However, we also observed that the biotic environment explained more variability in $\delta^{15}N$ than the abiotic one. This likely relates to the strong negative correlation between the foliar $\delta^{15}N$ and vegetation cover and species richness of the surrounding vascular plants (Table S2).

Within this study, the populations' elevational position, rather than TDUGS or PDUGS, altered the foliar $\delta^{13}C$ value, as the association between $\delta^{13}C$ and the former exceeded that between $\delta^{13}C$ and the latter (Tables 3, 4, Figs 3, 5). The plants occurring at lower elevation discriminated more against ^{13}C (i.e. they are characterized by more negative $\delta^{13}C$ values), whilst the plants occurring at higher elevation discriminated less against ^{13}C (i.e. they are characterized by less negative $\delta^{13}C$ values). When compared to the elevational mean of the continental populations (mean elevation ± standard deviation: 1989 ± 346 m a.s.l.), the Scandinavian populations were situated in significantly lower elevation (mean elevation ± standard deviation: 758 ± 276 m a.s.l.; ANOVA: lm: F= 86.06, $P < 0.001$) and are, therefore, exposed to higher atmospheric pressure reflected in increased discrimination against the heavier carbon isotope at lower elevations (Zhu et al. 2010). Our results are in accordance with the studies by Körner et al. (1991), Arroyo et al. (2009) and Zhu et al. (2010) who also reported increased discrimination towards the poles.

Relationship between leaf eco-physiological traits and traits important for dispersal and reproduction

We found several positive associations between the plants' foliar N content and foliar $\delta^{15}N$ values, and the stem height and capitulum size of continental European diploid and triploid plants, but no such pattern was found in Scandinavian plants. These relationships corroborate the general pattern of the increased nutrient requirement for production of reproductive organs (Sinclair 1975; Mattson Jr 1980; Reekie and Bazzaz 1987). In our study, Scandinavian plants, when compared to continental European plants, produced significantly fewer florets (i.e. ovules) per capitulum (mean ± SD of ovules: 2x: 75 ± 28; continental European 3x: 84 ± 37; Scandinavian 3x: 60 ± 29; ANOVA: lm: F= 10.21, $P < 0.001$). The lower N availability at high latitudes (Shaver et al. 1986; Bret‐Harte et al. 2002; Sitch et al. 2003), together with the presumably higher metabolic costs and demand of N and P to build DNA of polyploids compared to diploids (Lewis Jr 1985; Sterner and Elser 2002; Leitch and Leitch 2008), thus could have a negative impact on the number of ovules produced per inflorescence of Scandinavian plants.

Eco-physiological differentiation of Hieracium alpinum *plants from Scandinavia*

The differences in the eco-physiological leaf traits between the cytotypes and the associations with the populations' abiotic and biotic environment found in our study varied depending on whether Scandinavian populations were included in the analyses or not. The plants from Scandinavia considerably differed from the continental European plants mainly in foliar N content, which was in average 21% lower in the former group. The obvious drop of the N contents, together with the 2% decrease of foliar C contents, resulted in 28% higher C/N ratios in Scandinavian plants. In addition, the $\delta^{13}C$ values were on average about 3% lower in Scandinavian plants when compared to the continental plants. As discussed above, the latter pattern was likely caused by the increased partial CO_2 pressure at low elevations such as in Scandinavia (Körner et al. 1991; Arroyo et al. 2009; Zhu et al. 2010). Though the alpine habitats of *H. alpinum* across its distributional range are, at first glance, are characterized by a similar physiognomy, alpine habitats in Scandinavia have more open vegetation with a lower proportion of vascular plants, but higher proportion of bryophytes and lichens than alpine habitats in continental Europe (Hartmann et al. 2017). Furthermore, the northerly situated Scandinavian sites are exposed to a significantly lower cumulative amount of sun irradiation (SDUGS is lower about 21% in average) and precipitation (PDUGS is lower about 40% in average) during growing season than continental sites. Therefore, the high latitudinal position of Scandinavia is concomitant by the lack of energy input *per se* (SDUGS), which is also mirrored in the lowered net primary production (Iqbal 1983; Sitch et al. 2003). Such low productive sites in Scandinavia are characterized by a higher proportion of non-vascular plants, which have a lower biomass production and quality (less N) and thus slower decomposition rates (Hobbie et al. 2000). Lower productivity of alpine habitats in Scandinavia was mirrored not only in lowered foliar N and C contents but also in lowered investments in plant reproductive organs (number of ovules per capitulum). Interestingly, overall slower growth, smaller size of plant rosettes and smaller stem height of plants from Scandinavia when compared to plants from continental Europe have been observed in the greenhouse and common garden experiment (Hartmann et al. in prep.; Fig S8). This might suggest a genetically or epigenetically based adaptation to a less productive environment as discussed above (see also Scheepens et al. 2010 and Bauert 1996 for genotype-dependent response to environmental conditions).

Conclusions and further perspectives

Our study is likely among the first ones which have thoroughly documented intraspecific variation in foliar carbon and nitrogen contents, their stable isotope compositions and specific leaf area across the distributional range of an arcto-alpine species. Though we did not find the differences in most of the eco-physiological traits between two allopatric cytotypes of *Hieracium alpinum*, our results showed striking differentiation in the amount of foliar nitrogen and carbon contents between the alpine populations from Scandinavia and those from continental Europe. Because our preliminary results from the germination and greenhouse experiments have confirmed the distinctiveness of the Scandinavian populations (Hartmann et al. in prep.), altogether, these results might suggest different adaptation strategy of the populations from Scandinavia. We suppose that the altered ecophysiology and growth of Scandinavian plants reproducing strictly asexually could evolve either through directional selection acting at the level of clones or epigenetic modifications and that these changes should have occurred relatively recently, during or after postglacial re-colonization of Scandinavia from southerly situated glacial refugia (Hewitt 1999). In this context, it would be of particular interest to search whether alternate eco-physiology has been accompanied by structural and physiological changes in Scandinavian plants leading to better optimization of the light capture for a given leaf surface and hence photosynthetic capacity (Reich et al. 1997; Wellstein et al. 2017), which would be especially important under low sun irradiance coupled with low amount of available soil nutrients.

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Supporting information

Table S1. Mean values of three abiotic factors of sampling sites during the growing season (May-September) derived from the WorldClim2 model, and mean values and standard deviations of eco-physiological leaf traits in sexual diploid and asexual triploid populations of *Hieracium alpinum*. TDUGS—mean Temperature [°C], PDUGS—amount of precipitation [mm], SDUGS—amount of solar radiation [MJ m^{-2} day^{-1}]

Population information						***Abiotic factors***			***Leaf traits***					
Code	Region	Latitude	Longitude	Elevation [m]	Size	TDUGS	PDUGS	SDUGS	foliar C content [%]	foliar N content [%]	C/N ratio	δ^{15}N [‰]	δ^{13}C [‰]	SLA [mm^2 mg^{-1}]
sexual diploid populations														
H073	SC	45.380	23.511	2099	9	6.54	5.76	5.76		2.74 ± 0.44	16.30 ± 2.51	-6.48 ± 1.10	-28.40 ± 0.93	231.01 ± 21.06
H074	SC	45.407	25.481	2119	9	6.36	5.42	5.42		2.62 ± 0.32	17.24 ± 2.19	-5.47 ± 0.85	-28.31 ± 0.45	238.38 ± 21.74
H077	SC	45.590	24.634	1944	9	8.10	7.62	7.62		3.28 ± 0.70	13.74 ± 2.52	-3.69 ± 1.03	-29.31 ± 0.72	200.92 ± 64.58
C002	EC	46.765	22.795	1788	9	7.50	7.18	7.18		2.56 ± 0.62	18.40 ± 4.34	-2.92 ± 0.91	-28.88 ± 0.28	219.91 ± 26.10
H057	EC	47.386	25.538	1693	3	9.84	9.06	9.06		2.54 ± 0.30	17.92 ± 2.07	-3.71 ± 2.83	-28.98 ± 0.10	160.04 ± 28.09
C001	EC	47.581	24.802	1770	4	7.96	7.60	7.60		2.68 ± 0.51	17.13 ± 3.10	-2.79 ± 1.02	-28.93 ± 0.77	174.76 ± 26.15
H063	EC	48.252	24.231	1416	10	8.74	8.48	8.48		2.38 ± 0.43	19.06 ± 3.23	-5.87 ± 0.91	-29.07 ± 0.40	142.51 ± 24.06
H067	EC	48.832	22.977	1321	9	9.82	12.42	12.42		2.35 ± 0.26	19.04 ± 2.17	-4.96 ± 0.90	-28.97 ± 0.77	196.92 ± 20.46
H087	AP	43.956	17.735	2040	5	8.58	7.66	7.66		2.42 ± 0.46	18.72 ± 3.49	-1.32 ± 0.58	-28.15 ± 0.54	186.11 ± 28.66
H029	AP	45.171	5.975	2450	7	5.02	4.46	4.46		2.41 ± 0.28	18.64 ± 2.09	-7.11 ± 1.40	-28.52 ± 0.34	179.09 ± 25.12
H110	AP	45.370	7.107	2599	5	5.06	5.68	5.68		1.68 ± 0.10	25.83 ± 1.56	-6.63 ± 1.13	-28.26 ± 0.26	135.81 ± 8.61
H003	AP	46.255	8.010	2301	6	6.46	7.20	7.20		1.46 ± 0.26	30.56 ± 4.73	-8.38 ± 2.20	-28.07 ± 0.85	159.55 ± 11.70
H004	AP	46.570	8.408	2457	8	7.04	6.58	6.58		2.24 ± 0.31	20.06 ± 2.73	-7.18 ± 0.52	-28.09 ± 0.54	157.71 ± 12.13
H114	AP	47.123	10.186	2231	9	5.74	5.56	5.56		2.37 ± 0.37	19.39 ± 3.00	-6.32 ± 0.98	-29.06 ± 0.45	206.19 ± 33.83
H042	WC	48.886	20.129	1931	4	6.10	5.46	5.46		2.60 ± 0.38	17.38 ± 2.36	-2.40 ± 0.96	-27.34 ± 0.59	179.36 ± 25.28
Table S1 continued														
H033	WC	48.928	19.582	1993	6	7.82	14.50	14.50		2.69 ± 0.28	16.65 ± 1.76	-4.81 ± 1.22	-27.92 ± 0.54	217.65 ± 22.74
H047	WC	49.205	20.235	1936	10	5.66	4.90	4.90		2.41 ± 0.52	19.45 ± 4.59	-3.68 ± 2.59	-28.96 ± 0.80	176.14 ± 28.77
H032	WC	49.573	19.531	1704	7	7.22	6.20	6.20		2.94 ± 0.62	16.03 ± 3.90	-3.86 ± 0.91	-28.01 ± 0.38	168.85 ± 14.01
H094	SV	59.819	8.727	1270	11	7.48	5.82	5.82		2.02±0.33	22.19 ± 3.47	-6.03 ± 1.12	-28.97 ± 0.49	182.80±22.96
H095	SV	61.311	8.806	1097	8	7.24	7.74	7.74		2.05±0.47	22.00 ± 3.69	-6.90 ± 1.28	-28.68 ± 0.55	199.50±44.68

S03B	SV	64.860	13.253	596	10	8.72	8.36	8.36		1.94±0.17	22.68 ± 1.66	-4.64 ± 1.77	-30.25 ± 0.31	182.94±23.35
S03A	SV	64.870	13.276	733	2	8.08	7.54	7.54		2.37±0.34	18.86 ± 2.96	-3.37 ± 0.04	-30.87 ± 0.75	191.52±72.26
H099	SV	66.185	14.528	871	8	7.04	6.38	6.38		1.77±0.35	25.22 ± 4.36	-4.30 ± 0.81	-29.02 ± 0.31	173.30±22.38
H100	SV	66.545	15.333	729	8	7.30	7.86	7.86		2.11±0.35	21.22 ± 4.05	-4.88 ± 1.53	-29.10 ± 0.73	172.72±25.15
H101	SV	68.461	18.027	461	11	7.08	9.26	9.26		1.68±0.19	25.49 ± 2.60	-5.35 ± 1.72	-30.20 ± 0.35	230.36±31.14
H102	SV	69.054	20.735	561	5	6.98	8.18	8.18		1.71±0.09	25.75 ± 1.24	-4.63 ± 0.61	-29.64 ± 0.59	165.91±34.59
H103	SV	70.159	23.750	505	9	6.48	7.06	7.06		2.04±0.69	24.22 ± 8.93	-3.19 ± 1.42	-29.53 ± 0.87	173.71±31.07
2x					62	7.96±1.22	595.34±86.95	17.66±0.28		2.57±0.46	17.80 ± 3.04	-4.36 ± 1.83	-28.84 ± 0.68	192.50±42.13
3x					139	6.89±1.00	502.92±177.67	16.01±2.42		2.13±0.51	21.81 ± 5.04	-5.15 ± 2.11	-28.93 ± 0.95	182.75±33.50
CEU 3x					67	6.40±1.07	647.99±133.54	17.97±2.12		2.34±0.54	20.12 ± 5.08	-5.32 ± 2.49	-28.34 ± 0.71	177.68±30.93
SCV 3x					72	7.35±0.64	367.93±80.23	14.19±0.50		1.93±0.39	23.38 ± 4.49	-4.98 ± 1.69	-29.48 ± 0.80	187.60±35.32

Table S2. Results of parametric correlation tests (Pearson) between eco-physiological leaf traits and biotic variables of *Hieracium alpinum* across all sampled plants and in diploid (2x), Scandinavian triploid (SCV 3x) and continental European triploid (CEU 3x) plants.

		Dataset									
Leaf trait	Biotic variable	complete		2x		3x		CEU 3x		SCV 3x	
δ^{15}N	Richness	**-0.41**	***	**-0.5**	***	**-0.39**	**	**-0.52**	***	-0.12	*NS*
	E cover	**-0.3**	***	**-0.54**	***	**-0.24**	**	-0.17	*NS*	**-0.32**	**
	E1 cover	**-0.18**	*	**-0.35**	**	**-0.2**	*	-0.05	*NS*	**-0.28**	*
	E0 cover	-0.02	*NS*	-0.04	*NS*	0.04	*NS*	-0.14	*NS*	0.09	*NS*
foliar N [%]	Richness	-0.11	*NS*	**-0.25**	*	-0.07	*NS*	**-0.27**	*	0.14	*NS*
	E cover	0.04	*NS*	-0.07	*NS*	0.06	*NS*	-0.02	*NS*	0.11	*NS*
	E1 cover	**0.2**	**	0.06	*NS*	0.16	0.07	0.01	*NS*	0.08	*NS*
	E0 cover	**-0.22**	**	-0.14	*NS*	-0.16	0.08	-0.04	*NS*	-0.02	*NS*

Abbreviations: foliar δ^{15}N; foliar nitrogen content (foliar N [%]); richness – number of co-occurring vascular plant species (species richness); E cover – total vegetation cover, E1 – herb and dwarf shrub layer cover, E0 – bryophyte and lichen layer cover; NS – not significant $P > 0.1$); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; for marginally significant differences exact *P*-values are given.

Fig S1: Association between specific leaf area (SLA) and foliar N content of diploid (2x) and triploid (3x) plants of *Hieracium alpinum* and Scandinavian triploid (SCV 3x) and continental European triploid (CEU 3x) plants. Ablines represent the correlation between tested variables, independently for each of the two groups per plot (dashed) and across all individuals depict per plot (solid).

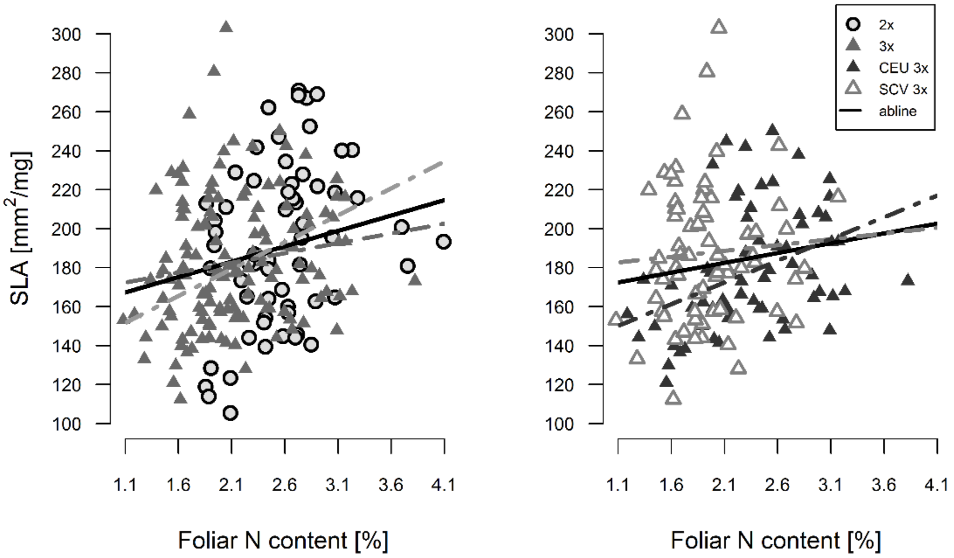
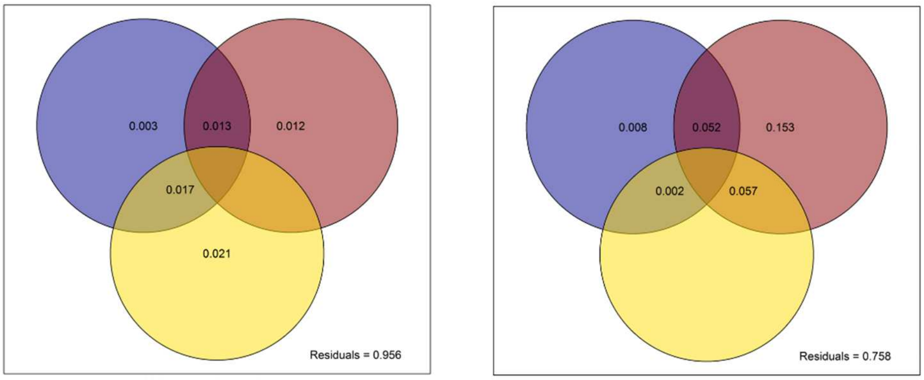


Fig S2 Results of the variance partitioning analyses. Proportions of explained variation of eco-physiological leaf traits of diploid and triploid *Hieracium alpinum* plants by the cytotype and the abiotic factors and their interactions. Tested fixed effects: Mean temperature during growing season (May – September; TDUGS); Amount of precipitation during the growing season (May – September; PDUGS); Amount of solar radiation during the growing season (May – September; SDUGS); Populations' elevational position (ELEV; solely for $\delta^{13}C$). Values < 0.0005 are not shown.

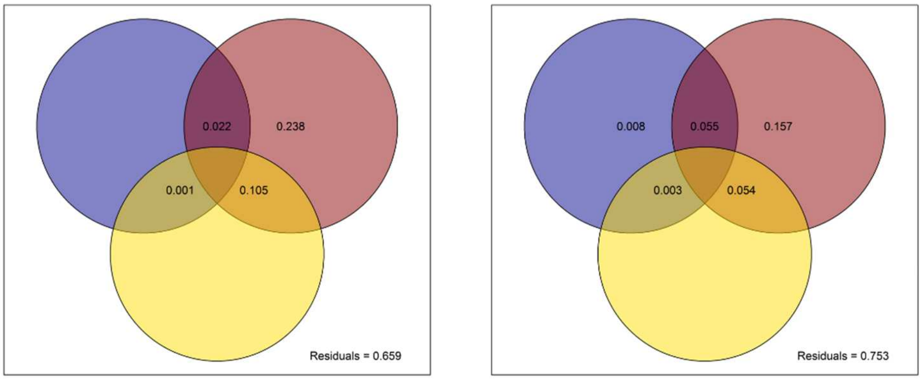
Specific leaf area (SLA)

Foliar N content [%]



Foliar C content [%]

C/N ratio



$\delta^{13}C$

$\delta^{15}N$

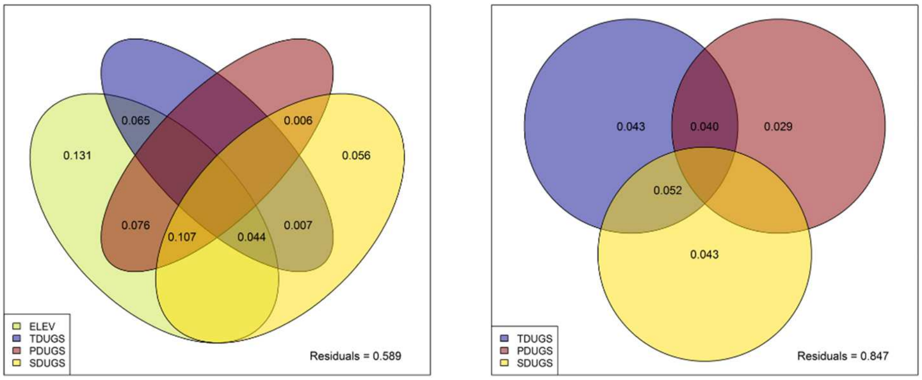


Fig S3 Results of the variance partitioning analyses. Proportion of explained variation of eco-physiological leaf traits of diploid and triploid *Hieracium alpinum* plants by the cytotype and the biotic factors and their interactions. Tested fixed effects: Number of co-occurring vascular plant species (Richness); cover by herb and shrub layer (E1 cover) and cover by bryophyte and lichens layer (E0 cover). Values < 0.0005 are not shown.

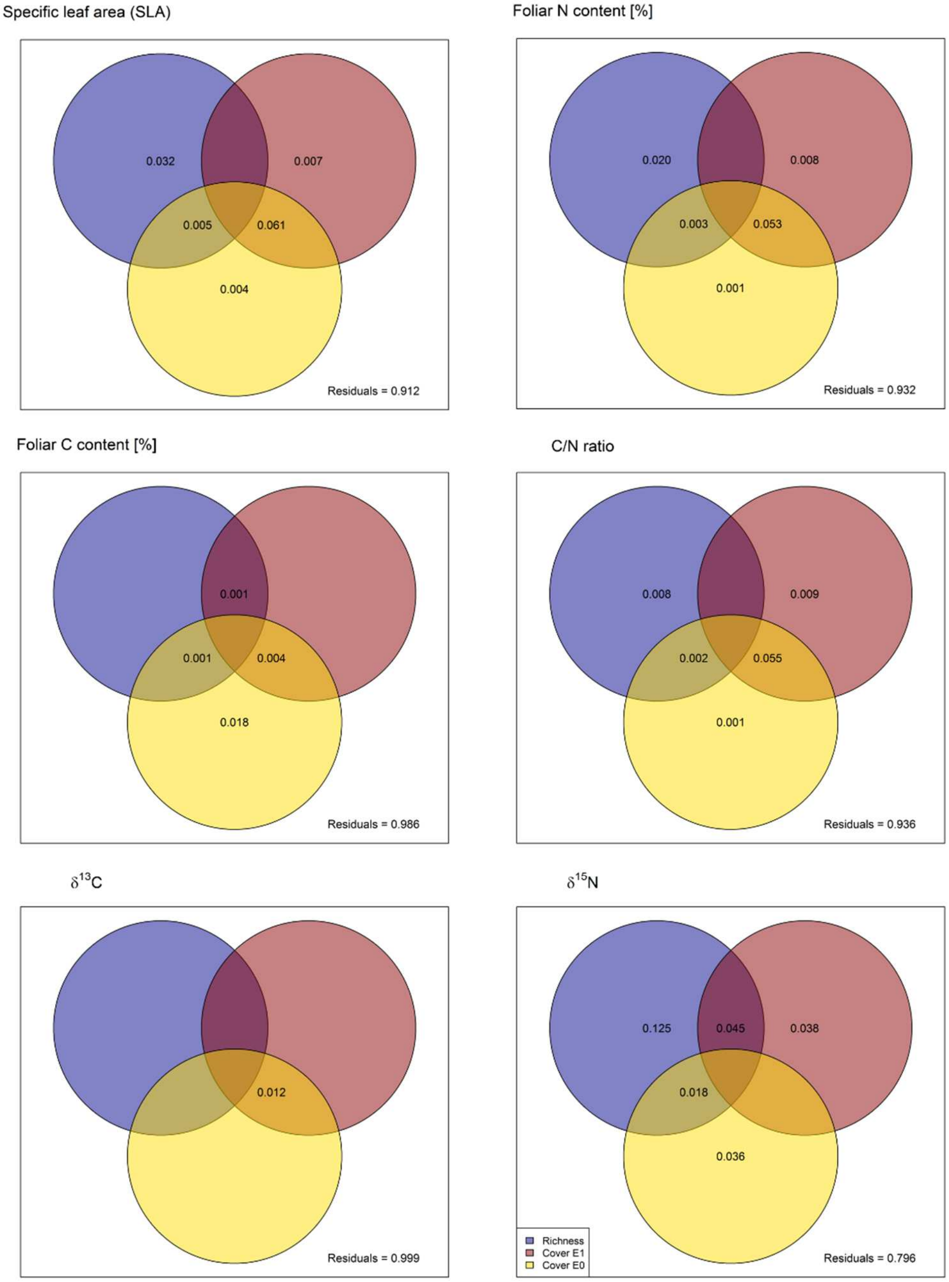


Fig S4 Latitudinal gradients in eco-physiological leaf traits of diploid (2x) and triploid populations of *Hieracium alpinum*. Size of the data points correspond to level of referring population mean. Foliar carbon content; Foliar carbon to nitrogen ratio (C/N ratio); Specific leaf area (SLA); Foliar δ^{15}N; Foliar N content; Foliar δ^{13}C.

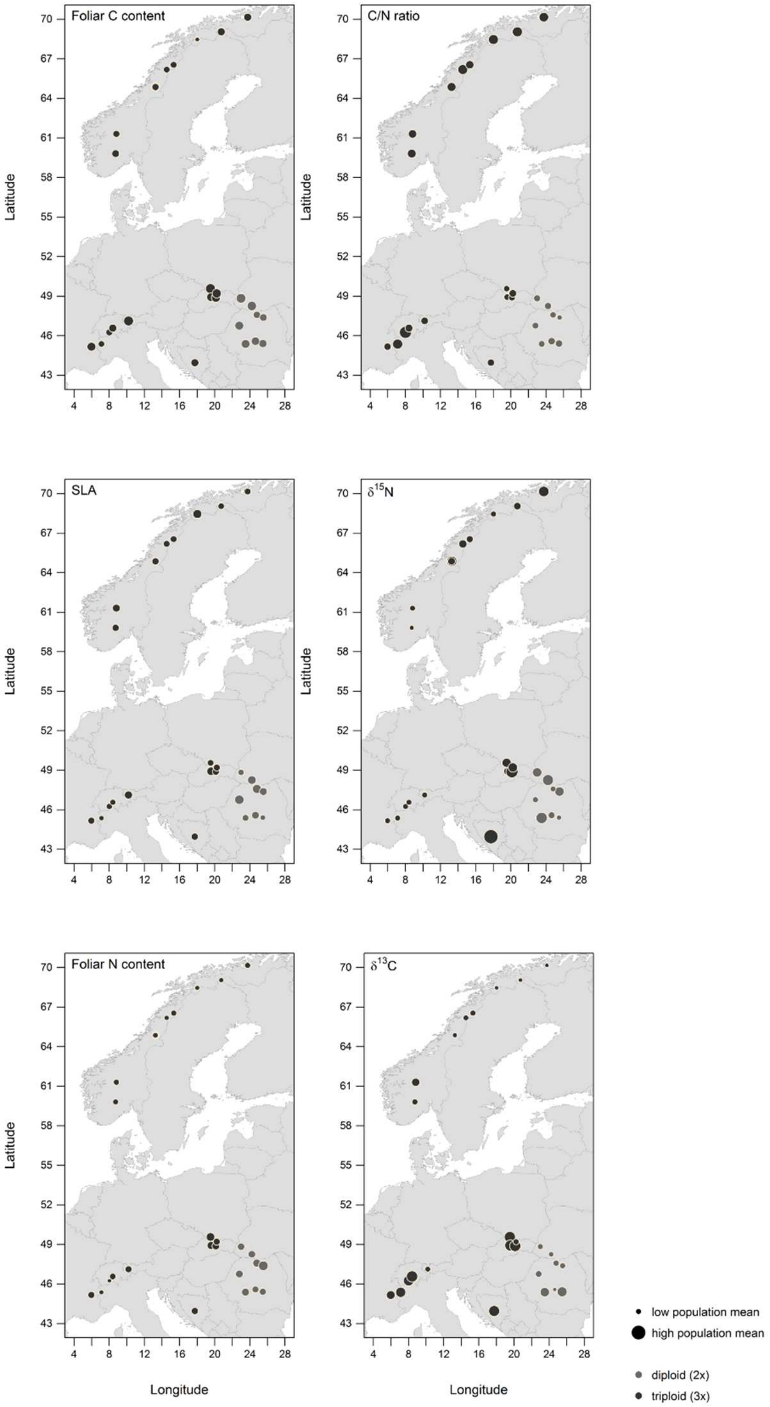


Fig S5: Boxplots of eco-physiological leaf traits in diploid and triploid populations of *Hieracium alpinum* sorted by latitudinal position. One boxplot represents one population; dashed lines represent average across all populations of the diploid, continental triploid and Scandinavian triploid range, respectively. Specific leaf area (SLA); foliar nitrogen content; foliar carbon content; foliar carbon to nitrogen ratio; foliar δ ^{13}C; foliar δ^{15}N.

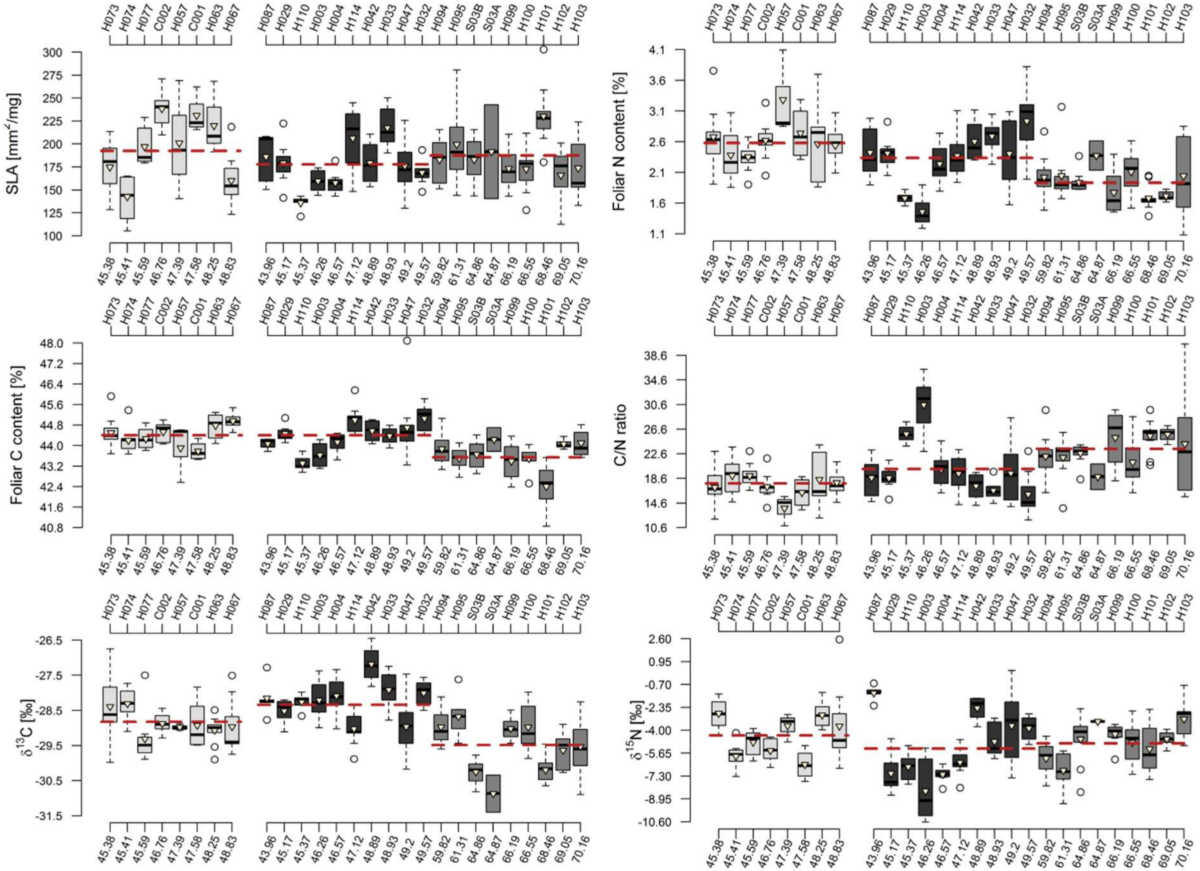


Fig S6. Boxplots of local abiotic environment in diploid (2x) and triploid (3x) populations of *Hieracium alpinum* and Scandinavian triploid (SCV 3x) and continental European triploid (CEU 3x) populations. Abiotic environment abbreviations: TDUGS – Mean temperature during growing season (May – September); PDUGS – Amount of precipitation during growing season (May – September); SDUGS – amount of solar radiation during the growing season (May – September). Triangles indicate mean values. Only statistically significant differences are displayed ** $P < 0.01$; *** $P < 0.001$.

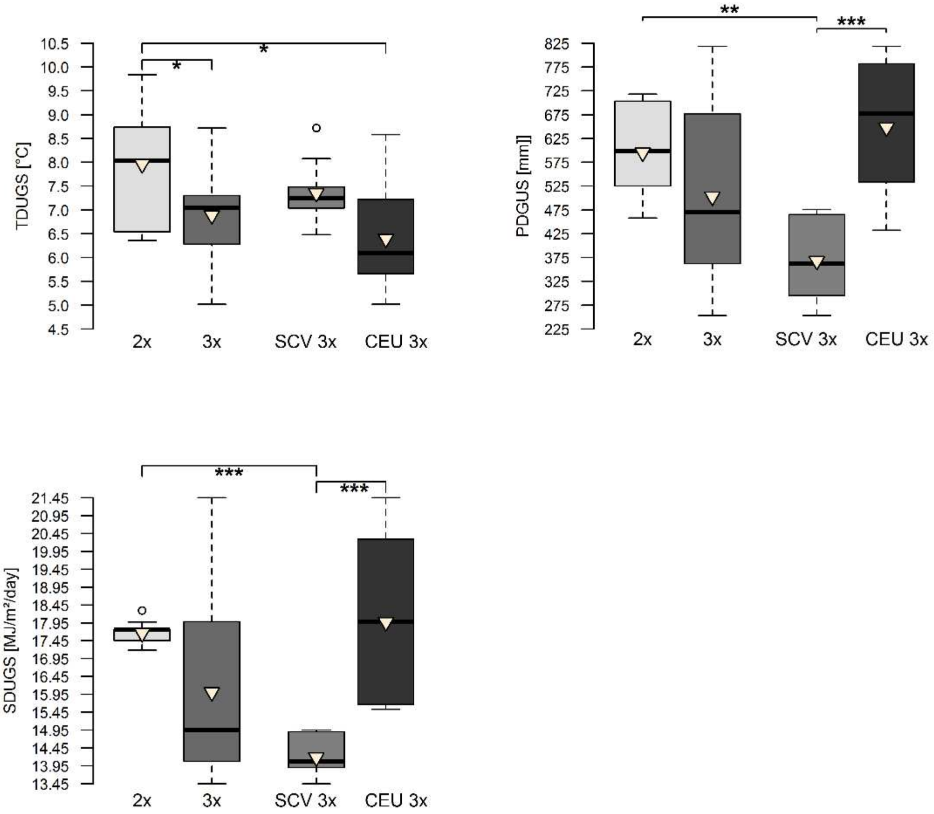


Fig S7: Associations between eco-physiological leaf traits of diploid (2x), central European triploid (CEU 3x) and Scandinavian triploid (SCV 3x) populations of *Hieracium alpinum* and traits important for dispersal (height of inflorescence; a, b) and reproduction (capitulum size; c, d), both assessed at the level of individual plants. Overall associations are indicated by dashed lines. Foliar nitrogen content (foliar N [%]); foliar δ^{15}N [‰].

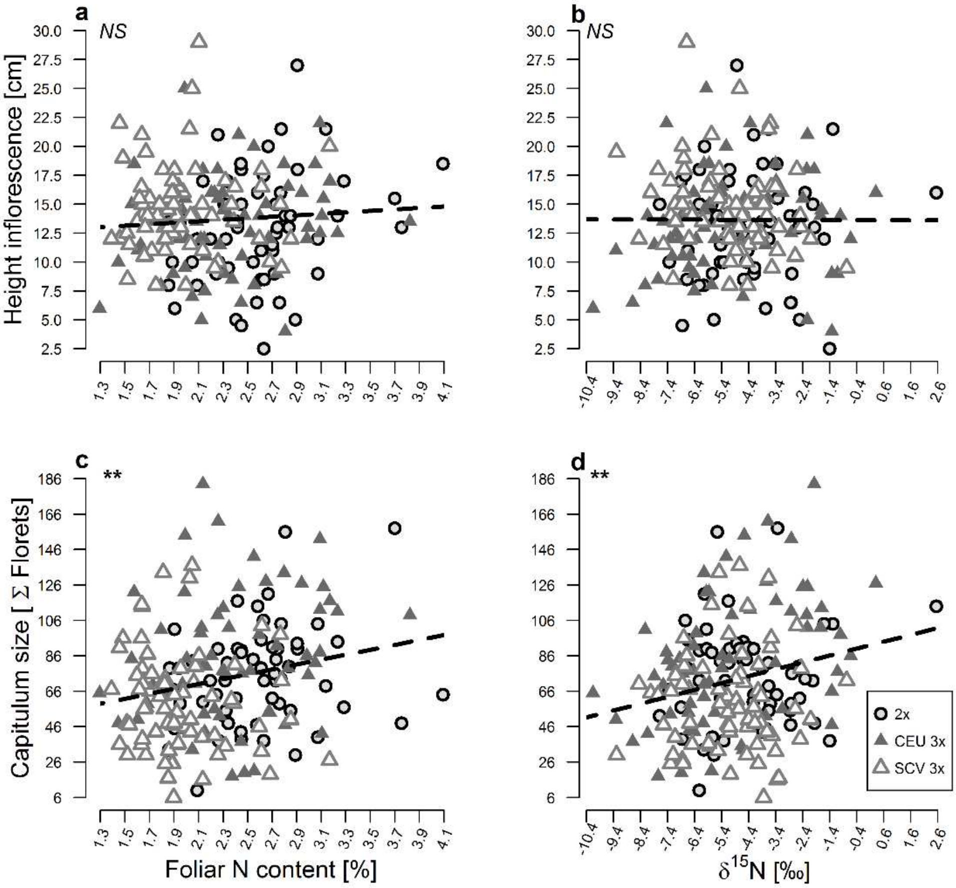


Fig S8. *Hieracium alpinum* plants of the same age in outdoor pots (growing in the same soil) in the botanical garden of Průhonice, the Czech Republic. The offspring of Scandinavian plants in the front is significantly smaller compared to offspring of other plants in the back, irrespective of the cytotype. (Photo credit: M. Hartmann; Průhonice 2017)

PAPER III

Mráz, P.; Zdvořák, P.; **Hartmann, M.**; Štefánek, M.; Chrtek, J.: Can obligate apomixis and more stable reproductive assurance explain the distributional successes of asexual triploids in *Hieracium alpinum* (Asteraceae)?
resubmitted to *Plant Biology*

Photo: Capitulum of *Hieracium alpinum* with not well-developed seeds (Photo Credit: P. Mráz)

Can obligate apomixis and more stable reproductive assurance explain the distributional successes of asexual triploids in *Hieracium alpinum* (Asteraceae)?

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Abstract

Though reproductive assurance has been suggested to be one of the most important factors shaping the differential distributional patterns between sexuals and asexuals (geographical parthenogenesis), it has only rarely been studied in natural populations of vascular plants with autonomous apomixis. Moreover, there are almost no data concerning the putative relationship between the level of apomictic *versus* sexual reproduction on one hand, and reproductive assurance on the other. We assessed the level of sexual *versus* apomictic reproduction in diploid and triploid plants of *Hieracium alpinum* across its distributional range using flow cytometric analyses of seeds, and compared the level of potential and realized reproductive assurance between both cytotypes under the field and greenhouse conditions. Flow cytometric screening of embryos and endosperms of more than 4,100 seeds showed that diploids produced solely diploid progeny sexually, while triploids produced triploid progeny by obligate apomixis. Potential fruit set was fairly the same in diploids and triploids from the field and the greenhouse experiment. While under the pollination limited environment in the greenhouse apomictic triploids had considerably higher realized fruit set than sexual diploids, there was no significant difference between cytotypes under natural conditions. In addition, sexuals varied to significantly greater extent in realized fruit set than asexuals under both, natural and greenhouse conditions. Our results indicate that triploid plants reproduce by obligate apomixis assuring more stable and predictable fruit reproduction when compared to sexual diploids. This advantage could provide apomictic triploids with a superior colonization ability mirrored in strong geographical parthenogenesis pattern observed in this species.

Keywords: Apomixis, breeding system, FCSS, flow cytometry, geographical parthenogenesis, polyploidy, reproductive assurance, fruit set.

Introduction

Geographical parthenogenesis (GP thereafter) is the geographically distinct distribution of asexually reproducing plant and animal species and their sexual relatives (Vandel 1928). According to GP, asexuals have often larger distributional ranges which are shifted to higher latitudes and altitudes than those of sexuals. Moreover, asexuals tend to occur more frequently in marginal and extreme habitats like recently de-glaciated areas or deserts than sexuals do (e.g. Bierzychudek 1985, Hörandl 2006). The distributional success of asexuals has attracted the attention of evolutionary biologists as it may provide a clue for understanding the advantages and disadvantages of sexual reproduction (Bell 1982). Among several hypotheses potentially underlying the GP patterns in plants (reviewed in Hörandl 2006), the advantage of uniparental reproduction relying on greater reproductive assurance of asexuals has been suggested as one of the most crucial (Hörandl 2006).

In angiosperms, asexual seed reproduction – apomixis (agamospermy) – is based on the autonomous formation of the embryo either from the somatic cell of the nucellus (apospory) or the megaspore mother cell (diplospory), or from sporophytic tissues (integumentary adventitious embryony) (Asker & Jerling 1992). In all of these cases, the progeny have the same set of maternal chromosomes and they are genetic clones of the seed parent as recombination (mixis) is completely circumvented (Asker and Jerling 1992). Apomixis can be either fully autonomous, i.e. formation of endosperm does not require the pollination of central nucleus (diplospory and non-pseudogamous apospory), or pseudogamous, when this requirement is needed (pseudogamous apospory and adventitious embryony) (Asker and Jerling 1992).

Because of uniparental reproduction, apomictic plants, similarly to self-compatible colonizers (Baker 1955), are theoretically capable of establishing a new population with only one individual. In contrast, their sexual relatives are often strictly self-incompatible, i.e. for successful seed production they need the presence of a compatible partner (Asker and Jerling 1992). This might be a great challenge especially in small founder populations when compatible partners should be sufficiently close together spatially and temporally (Baker 1955). Furthermore, autonomous apomicts (diplosporic and non-pseudogamous aposporic) are fully independent also from the presence and activity of pollinators. Considering the unpredictable nature of the pollination environment, such as, for example, limited number and activity of pollinators due to unfavorable weather conditions or competition for pollinators with other co-occurring plant species (Arroyo et al. 1985, Totland 1994, Vices and Bosch 200, Kalisz and Vogler 2003), this independence is even more relevant, as pollen limited seed sets have been commonly observed (Burd 1994, Ashman et al. 2004, Garcia-Camacho and Totland 2009). Therefore, the independence from pollinator agents might provide autonomous apomicts with an ultimate demographic advantage through greater and more stable reproductive assurance when compared to their sexual relatives. Indeed, 34 of 38 cases of GP patterns were recorded in the family of Asteraceae, which is characterized by prevailing autonomous apomixis (Hörandl et al. 2008).

Given its indisputable ecological importance, the reproductive assurance advantage confined to apomictic plants has surprisingly received very little attention. We are aware about two studies only, which quantitatively compared the reproductive success between asexuals with autonomous apomixis and their sexual relatives under natural conditions. These studies have provided mixed support for higher reproductive assurance in apomicts. O'Connell & Eckert (1999) did not find any differences in mean seed sets between the natural sexual and apomictic populations of *Antennaria parlinii* (Asteraceae), but this pattern changed in pollinator-free greenhouse when sexuals set seeds only after manual cross-pollination. In contrasts, van Dijk (2007) reported consistently higher seed sets in triploid apomictic dandelions (*Taraxacum* Sect. *Ruderalia*; Asteraceae) when compared to diploid sexuals in three natural populations in the Netherlands for three consecutive years.

Because of the extreme scarcity of studies focused on the reproductive assurance differences between sexual and asexual plants with autonomous apomixis, we have performed a large scale assessment of seed sets in 35 natural populations of *Hieracium alpinum* (Asteraceae) across the distributional range of species. Specifically, we asked whether the colonization success of the apomictic triploid cytotype (Fig. 1; Mráz et al. 2009) could be due to a higher and more stable realized reproductive assurance, i.e. production of well developed seeds in a capitulum, when compared to sexual diploid cytotype. Furthermore, because the apomixis is tightly linked with polyploidy (Asker & Jerling 1992), a phenomenon which can trigger phenotypic and physiological changes, we assessed whether potential reproductive output expressed as the total number of florets per flower head differ between diploids and triploids. In addition to natural conditions, both realized and potential fruit sets were inferred also in plants cultivated under the similar conditions in the greenhouse simulating pollinator-restricted environment. The reproductive assurance advantage of asexuals might be lowered in facultative apomicts when compared to obligate apomicts as the former combine sexual and apomictic reproductive strategies within the same individual. Therefore, we quantitatively evaluated the mode of reproduction in 838 plants using flow cytometric seed screening analyses.

Hieracium alpinum is a suitable model species for such an assessment for several reasons. The species consists of two cytotypes, diploid (2n = 2x = 18) and triploid (2n = 3x = 27) which differ in their mode of reproduction (Chrtek 1997). While diploids reproduce strictly sexually and need a compatible partner for successful pollination and fertilization (sporophytic self-incompatibility system) (Chrtek 1997; Mráz 2003), triploids produce seeds asexually from a mitotically derived unreduced embryo sac (Skawińska 1963). The meiosis and syngamy are completely omitted and the endosperm develops autonomously without pollination of the central nucleus (diplospory of *Antennaria* type; Skawińska 1963). Both cytotypes differ also in their male function. While diploids produce a lot of homogenously sized pollen, triploids are usually pollen sterile, only rarely form a small amount of heterogeneous sized pollen grains (Chrtek 1997; Slade & Rich 2007; Mráz et al. 2009). Vegetative reproduction, which level is very similar between both cytotypes (generalized linear mixed-effect model with Poisson distribution, $z = 1.438$, $p = 0.15$), is rather limited and is restricted to the production of accessory rosettes developing from hypocotyl meristems arising at the base of main rosette (Mráz et al. unpubl.). The plants have usually only one unbranched stem bearing one capitulum (flower head) – inflorescence, which is composed of several tens of florets. Each floret has one ovule from which one achene (dry, single seeded fruit) can be formed. If accessory rosettes are present on a plant, then usually each of them has one stem. Importantly, the species shows a typical pattern of GP; sexual diploids occur only in the Eastern and Southern Carpathians (Romania, Ukraine) while triploids occupy the remaining and considerably larger part of the species range which includes Greenland, Iceland, Scotland, Scandinavia, polar regions of European Russia, the Ural Mts, and several mountains ranges in continental Europe (the Alps, the Western Carpathians, the Sudetes, the Vosges, the Harz and the Vranica Planina) (Fig 1; Mráz et al. 2009). Both cytotypes form ploidy-uniform populations, which exhibit non-overlapping – allopatric distribution (Mráz et al. 2009). Previous investigations using amplified fragment length polymorphism markers (AFLPs), internal transcribed spacers (ITS1 and ITS2), three cp DNA loci and more than 1,000 nuclear exons revealed that triploid plants are of autopolyploid origin (Mráz et al. in prep.). Based on the genetic structure and allopatric distribution of cytotypes, Mráz et al. (2009) hypothesized that despite an autopolyploid origin and very close relationship to diploids, asexual triploids are not direct descendants of contemporary diploid populations, but are rather remnants of extinct diploid lineages which had occurred outside of the current diploid range. This scenario thus suggests that extant triploid genotypes have had superior fitness and/or colonization ability than the sexual diploids, which have been completely replaced within the current range of triploids. Therefore, increased and more stable reproductive assurance of asexuals, which are completely independent on pollinators and available mating partners might provide them an ultimate fitness advantage when compared to sexual lineages.

In our study we aim to test the following hypotheses: (i) Based on previous embryological, castration and isolation experiments in *Hieracium alpinum* (Skawińska 1963; Chrtek 1997; Mráz 2003), but also on differences between diploids and polyploids in their extent of morphological and genetic variation (Shi et al. 1996; Chrtek 1997; Štorchová et al. 2002; Chrtek & Plačková 2005), we suppose that diploid sexuals reproduce solely sexually, while triploid asexuals reproduced mostly, if not completely, apomictically. (ii) Because of differences in ploidy level, we expect that both cytotypes will differ in their potential fruit sets – the number of florets per capitulum, and that this difference will be more pronounced under the uniform greenhouse conditions as the possible environmental effect will be removed. (iii) We expect that, due to their dependency on availability of compatible mating partners and pollinators, sexual diploids will have reduced realized fruit sets (% of well developed fruits per capitulum) when compared to asexual triploids under natural conditions and this pattern will be magnified in the greenhouse with more restricted conditions for pollination. (iv) As a consequence of stochastic pollination environment and availability of mating partners, we expect that the realized fruit set will vary in greater extent in sexuals under field conditions and even more in the greenhouse experiment than in asexuals.

Material and Methods

Field sampling

In the summers of 2014 and 2015, we visited 35 populations throughout the distributional range of the species, out of which four populations were visited repeatedly in both years (Table 1, Fig. 1). At each site we sampled ripe achenes from fully open and complete and visually not damaged capitula collected on 22 plants (= seed families) in average (ranged between 1 and 57 per site). The sampling size varied according to the number of suitable plants (Table 1).

Sampling was conducted in two crossed-over linear transects (each with the same number of plants), if possible, and with 2 m as a minimum distance between the plants. In the event of limited number of suitable plants (i.e. with fully mature achenes), we collected also the plants with not fully matured capitula but which accomplished completely flowering stage, i.e. after pollination and fertilization stage. These plants including rhizomes and roots were dug up, potted immediately into the small 0.1 L pots and kept until the capitula fully matured.

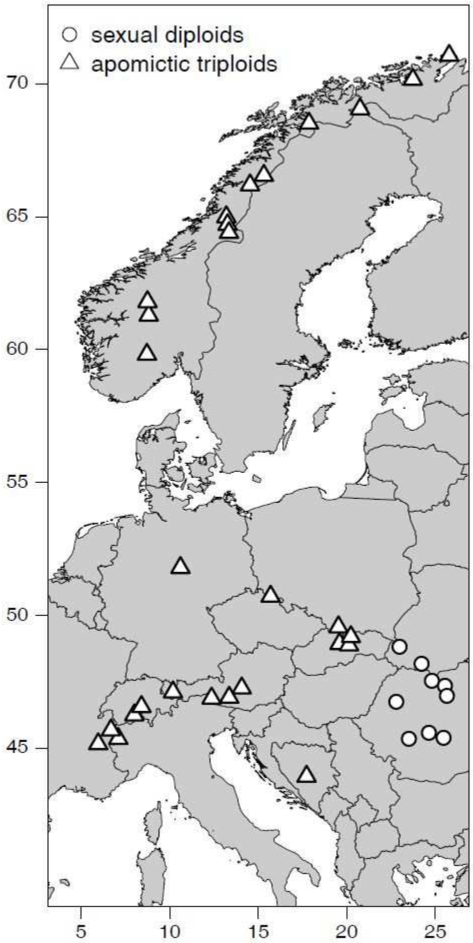

Fig. 1 Map of sampling sites of *Hieracium alpinum* populations used for assessments of reproduction modes, ploidy levels and reproductive assurance.

Table 1. List of visited populations of *Hieracium alpinum* assessed for the mode of reproduction and the level of reproductive assurance. Abbreviations: Pop – population code; site – locality in abbreviated form; lat / long – N latitude / E longitude; Nrep – number of plants tested for mode of reproduction; Nsee – number of seeds analysed by flow cytometry; Nass – number of plants assessed for reproductive assurance in the field in 2014 (in 2015) / in the greenhouse experiment; Mean seed set – mean percentage of well-developed seeds per capitulum ± standard deviation assessed in 2014 (2015) in the field / in the greenhouse. Countries abbreviations: AT – Austria, BA – Bosnia and Herzegovina, CH – Switzerland, CZ – Czechia, DE – Germany, FR – France, NO – Norway, RO – Romania, SK – Slovakia, UA – Ukraine. † denotes those populations from where the seedlings and / or repotted adult plants were assessed for their ploidy level. NA – not assessed.

Pop	Site	lat / long	alt	Nrep	Nsee	Nass	Mean seed set ± SD [%]
Diploid sexual populations							
H073†	RO, Parăngul Mic	45.38 / 23.51	2100	84	421	24 (57) / 40	57.5 ± 18.8 (85.4 ± 9.4) / 4.6 ± 17.4
H074†	RO, Babele	45.41 / 25.48	2120	52	257	37 (57)	18.6 ±15 (81.6 ± 16.1)
H077†	RO, Arpaşu Mic	45.59 / 24.63	1960	63	315	14 (40) / 39	50.3 ± 19.3 (85.8 ± 11.4) / 2.9 ± 10.3
C002†	RO, Mt. Vlădeasa	46.76 / 22.79	1825	34	165	37 / 17	64.4 ± 22.1 / 3.6 ± 5.8
H057†	RO, Pietrosul Bogolin	47.38 / 25.54	1690	52	259	42 (24) / 28	62.6 ± 29.8 (69.0 ± 21.8) / 2.3 ±5.4
HBRO	RO, Pietrosul Brostenilor	47.38 / 25.54	1730	17	84	16	70.7 ± 16.0
C001†	RO, Şaua Gargalău	47.58 / 24.80	1770	34	117	45	71.1 ± 17.3
H063†	UA, Stih	48.25 / 24.23	1420	19	52	18 / 24	91.3 ± 5.3 / 0.9 ± 1.8
H067†	UA, Pikuï	48.83 / 22.99	1380	1	5	15	26.6 ± 31.7
Triploid apomictic populations							
H087†	BA, Nadkrstac	43.95 / 17.73	1980	14	80	15 / 15	54.5 ± 16.3 / 77.6 ± 9.9
H029	FR, Petit Doménon	45.16 / 05.95	2390	8	45	5	45.3 ± 20.6
H110†	FR, Roc des Pareis	45.37 / 07.10	2570	56	297	1 (43) / 8	56.8 (48.6 ± 18.0) / 56.1 ± 17.5
H111†	FR, Cormet de Roselend	45.69 / 06.69	2010	8	40	1 / 1	75.5 / 70.1
H003†	CH, Simplonpass	46.26 / 08.01	2300	16	83	13	68.3 ± 16.1
H004†	CH, Furkapass	46.57 / 08.41	2460	39	225	37 / 2	65.0 ± 14.6 / 67.9 ± 2.8
H020†	AT, Moseralm	46.89 / 12.37	2360	13	80	5	73.3 ± 9.2
H022†	AT, Reißeck Hütte	46.92 / 13.36	2370	2	10	3	24.7 ± 9.3
H114	AT, Arlbergpass	47.12 / 10.19	2280	31	176	32 / 7	51.0 ± 30.1 / 83.7 ± 13.8
H026†	AT, Sölkpass	47.27 / 14.08	1690	2	10	4	73.5 ± 10.8
H042†	SK, Kráľova hoľa	48.89 / 20.13	1830	18	95	12 / 17	63.9 ± 15.6 / 68.2 ± 11.7
H033†	SK, Chopok	48.94 / 19.59	1960	19	103	16	45.1± 16.8
H047†	SK, Veľká Svišťovka	49.20 / 20.23	1930	26	143	25 (14) / 9	20.7±19.4 (67.8±9.3) / 87.0 ± 6.2
H032	SK, Babia hora	49.57 / 19.53	1700	44	236	39 / 40	53.6 ± 14.6 / 85.4 ± 7.4
H082†	CZ, Studniční hora	50.72 / 15.72	1416	4	25	11 / 35	66.8 ± 15.6 / 86.1 ± 25.5
BROC	DE, Brocken	51.80 / 10.61	1080	bulk	25	NA	NA
H094†	NO, Gaustakane	59.82 / 08.72	1280	22	123	25 / 4	69.3 ± 11.8 / 79.3 ± 5.6
H095†	NO, Båtskardet stølane	61.29 / 08.82	1160	17	50	1	2.9

Table 1 continued

S001†	NO, Svarttjøna	62.43 / 09.72	1250	1	3	1 / 6	71.4 / 80.7 ± 10.7
S02A†	NO, Steinfjellet	64.87 / 13.26	720	3	14	1	85.7
S02B†	NO, Steinfjellet	64.84 / 13.27	840	5	11	7	80.4 ± 12.3
S003†	NO, Steinfjellet	64.86 / 13.26	590	15	68	18 / 23	80.1 ± 9.3 / 66.5 ± 15.0
H099†	NO, Hellerfjelet	66.19 / 14.54	770	28	140	16	80.9 ± 9.5
H100	NO, Polar Circle	66.55 / 15.33	680	21	56	8	60.5 ± 11.9
H101†	NO, Gáicajávrrit	68.51 / 17.87	400	30	165	35 / 28	81.1 ± 14.7 / 81.6 ± 12.5
H102†	NO, Storfjorfd	69.05 / 20.75	560	26	97	36 / 20	75.2 ± 14.3 / 77.8 ± 8.7
H103†	NO, Bigášjávrrit	70.16 / 23.73	410	18	52	39 / 7	50.6 ± 20.0 / 84.5 ± 7.6
NOK†	NO, Nordkapp	71.06 / 25.78	200	NA	NA	NA	NA

Greenhouse experiment

In the greenhouse experiment, which was focused on comparison of responses of selected traits between both cytotypes (Hartmann et al. in prep.), we used only a subset of populations and seed samples collected in the field (Table 1). From each of the 20 populations, we used seed samples of 4 (sometimes 3) seed families. The seeds were let to germinate. Sixteen cultivated plants (= replicates) per seed family originated from these seedlings were used in the greenhouse experiment. The plants were cultivated in 0.25 L pots filled with a mixture of peatbog soil, garden compost and siliceous sand in ratio 4 : 2 : 1 and were regularly fertilized by Kristalon Gold fertilizer (AGRO CS a.s., Czechia). The greenhouse experiment lasted from January to October 2016. The open capitula with ripen achenes were collected continuously from mid of May to the end of October. In order to ensure favorable conditions for the experiment during the summer period, the aeration windows of the greenhouse were open frequently allowing sporadic visits of different species of insects (Diptera, Hymenoptera, Lepidoptera; pers. observations). Because of aphids, whiteflies and trips infestation, all *Hieracium alpinum* plants were repeatedly treated with biological control agents (mite *Amblyseius cucumeris*, and wasps *Encarsia formosa* and *Aphidius colemani*) provided by Biocont Laboratory (Modřice, Czechia). Both, the occasional insect visitors and the biological control agents could therefore mediated pollination of flowering plants cultivated in the greenhouse.

The collected capitula from the field and the greenhouse, were stored at room temperature for one month during which the seed set assessment was conducted. After one month, the samples were transferred into the fridge and stored at 4 °C.

Potential and realized fruit set assessments

The potential fruit set was determined as a total number of fruits in a complete capitulum (i.e. flower like inflorescence in Asteraceae) regardless the state of fruits. The realized fruit set was determined as a proportion of well-developed fruits to all fruits per capitulum. The well-developed fruits were classified as those which were usually dark brown / black, of 'normal' size and full (inspected by dissecting needle). As aborted seeds we classified those which were usually whitish, of smaller / shrunken size, and soft (i.e. without any content). The sterility of aborted seeds was confirmed also by flow cytometry, which revealed the absence of nuclei belonging to either embryonic or endospermic tissues. In contrast, well-developed seeds showed clear peaks corresponding to nuclei from embryo and endosperm. Although the field sampling targeted the plants with fully developed and not-damaged or aborted capitula, during fruit set assessments, we found relatively high number of capitula having traces of infestation by seed-eating larvae of tephritid and anthomyiid flies. Such samples were noted and subsequently excluded from the analyses focused on differences in realized fruit set. The level of potential and realized fruit set was determined for 1331 plants collected in the field and 410 plants cultivated in the greenhouse experiment. In total 98,247 and 84,708 achenes were evaluated in field survey and the greenhouse experiment, respectively.

Flow cytometric analyses of fruits and seedlings

Five to ten mature achenes per mother plant representing in average 12% to 25% of well-developed fruits per mother plant and in average 22 mother plants (between 1 and 63) per population were analyzed using flow cytometry to assess the ploidy of offspring (embryos) and the reproduction pathways (sexual *versus* asexual) (Table 1). Cytometrical discrimination between both modes of reproduction in *Hieracium* s.str. using flow cytometrical seed screening analysis (FCSS) is very straightforward: sexually derived fruits in *Hieracium* have the ratio of ploidy of endosperm to the ploidy of embryo equal to 1.5 because of double fertilization (i.e. in diploid *H. alpinum*, embryo is diploid and endosperm is triploid), while in apomictic polyploid plants this ratio is always 2 (in triploid *H. alpinum* embryo is triploid and endosperm is hexaploid) because of mitotically derived embryo sac (Matzk et al. 2000; Fig. 2). Given the cytological homogeneity within populations and plants, respectively (reviewed by Mráz et al. 2009), we analyzed the fruits of each plant as a bulk sample of five achenes. In the cases where the peaks corresponding to the endosperm were very low or even missing, we analyzed five additional seeds per plant. In total, the ploidy of seed progeny and reproduction mode were assessed in 4127 achenes originated from 842 plants from 35 localities (Fig. 1, Table 1).

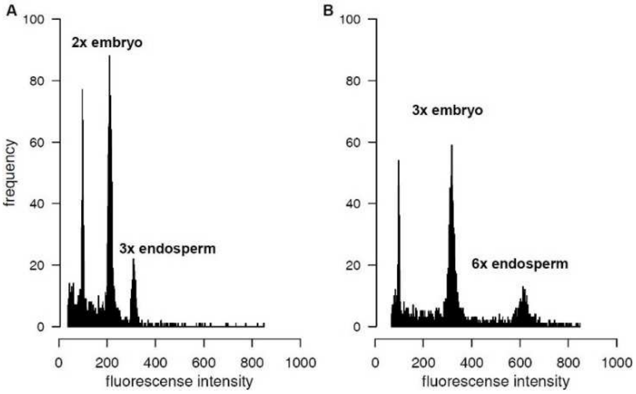

Fig. 2 Flow cytometric analyses of bulk samples consisting of five seeds of a sexually reproducing diploid (A, progeny of H73-17-5) and an apomictically reproducing triploid plant (B, progeny of H42-13-5) of *Hieracium alpinum*. Peaks of fluorescence intensities corresponding to ploidy levels of embryos and endosperms are depicted. The leftmost peaks in A and B show the relative fluorescence intensity of a standard plant, i.e. *Bellis perennis*.

In addition to the ploidy analyses of fruit samples, we detected the ploidy level for 209 seedlings from 177 plants originated from 30 populations, and 12 dig up plants from 7 populations (Table 1). We used the bulk samples consisting of small pieces of leaves from up to five seedlings originated from the same mother plant.

The fruit and leaf samples were prepared using two-step procedure (Otto 1990; Doležel & Göhde 1995) with co-chopped leaves of *Bellis perennis* as internal standard, 4,6-diamino-2-phenylindole (DAPI) as a fluorescent stain and using Partec Cyflow instrument equipped with a HBO lamp as ploidy level analyzer. Histograms were accumulated at a flow rate of approximately 10–30 particles per second for a total count of 1500 for seedlings / adult plants and 3000 nuclei at minimum for seed samples, respectively. The mean values of coefficients of variation of fluorescent intensities were as follows: 3.06% for internal standard, 3.17% for embryos, 2.54 for endosperms, 1.73 for seedlings and 3.74 % for adult plants. The ploidy level of samples was inferred as a relative position of the sample G1 peak to that of the internal standard.

Statistical analyses

Statistical analyses were performed in two ways. Firstly, we compared the fruit sets (potential and realized) between the two modes of reproduction (sexual *versus* asexual) separately for the plants sampled in the field and the plants sampled in the greenhouse experiment. The effects of ploidy or mode of reproduction on potential or realized fruit sets, respectively, were tested using linear mixed effect models (LMMs) and *lme* function implemented in the {nlme} package (Pinheiro et al. 2017) within the R environment (R Core Team, 2014). The models included potential (square-root transformed) and realized (arcsin square-root transformed) fruit sets per capitulum as response variables, and ploidy or mode of reproduction as explanatory variables. The population was treated as a random term. Because of violation of homogeneity of variance assumption, we used *varIdent* function implemented in {nlme} to account for different levels of variance between ploidies and among populations. Since digging and presence of seed herbivory had significantly negative effects on realized fruit sets in the capitula of plants sampled in the field (t-tests, results not shown), these capitula were removed from the analyses.

Secondly, we compared both fruit set variables between plants – seed families, which were sampled in the field and which progeny was used in the greenhouse experiment with the aim to test for the effect of plant origin (field *versus* greenhouse) and its interaction with ploidy/mode of reproduction. Because of very unbalanced design of this comparison which encompassed only one plant per seed family from the field on one hand, and up to 6 flowering plants (= replicates) per seed family in the greenhouse experiment on the other, we averaged both potential and realized fruit set per seed family for plants from the greenhouse experiment. We applied LMM using *lmer* function with the identity link function for normally distributed data and the likelihood ratio test (LRT, comparing a fully fitted model to the model from which the tested term was removed) to test the effect of ploidy level, origin of plants (field *versus* greenhouse) and their interaction on potential fruit set. We used LMM and *lme* function to test the effect of mode of reproduction, origin of plants and their interaction on realized fruit set. In this model, we accounted for different levels of variances between ploidies and plant origins using *varIdent* function. The population was treated in both cases as a random term.

We calculated the coefficient of variation of realized fruit set as a proxy of unpredictability of seed production in sexual and asexual plants, respectively, and separately for the field and greenhouse data. In addition, for the field dataset, which showed no difference in mean values in realized fruit sets between sexuals and asexuals, we calculated also the variance and tested it using *var.test* function implemented in R.

Finally, we used Spearman correlation tests to search whether realized fruit sets could be influenced by the size of capitulum (potential fruit set).

Results

Breeding systems

In the fruit samples collected from the Eastern and Southern Carpathians populations, solely diploid embryos (average ratio of the fluorescence intensities of peaks corresponding to embryo to the standard was 2.15 ± 0.04) and triploid endosperms (average ratio of the fluorescence intensities of peaks corresponding to endosperms to the standard was 3.19 ± 0.06) were found. Average ratio of the fluorescence intensities of peaks corresponding to endosperms to fluorescence intensities of the peaks corresponding to embryos was 1.48 ± 0.02, thus very close to the expected ratio 1.5 suggesting strict sexual reproduction. Similarly, among 61 cytometrically analysed seedlings, which originated from the samples collected in the diploid range, all were diploid, but one (H57_11_2), which was likely hyperdiploid with $2n$ = ca 19-20 instead of $2n = 18$ (the ratio of fluorescence intensity of the sample to the fluorescence intensity of standard was 2.33, while the ratio of remaining diploid seedlings of the same mother plant was 2.16; Fig. S1).

In the samples collected in the remaining geographical range, we solely found seeds with triploid embryos (average ratio of the fluorescence intensities of peaks corresponding to endosperms to the standard was 3.17 ± 0.05) and hexaploid endosperms (average ratio of the fluorescence intensities of peaks corresponding to endosperms to fluorescence intensities of the peaks corresponding to the standard was 6.25 ± 0.12). Average ratio of the fluorescence intensities of peaks corresponding to endosperms to fluorescence intensities of the peaks corresponding to embryos was 1.97 ± 0.03. This corroborates our expectation about the obligate apomixis in the triploid cytotype. We found solely triploid seedlings ($n = 148$, average ratio of the fluorescence intensities of samples to the fluorescence intensities of internal standard was 3.19) originated from plants sampled in the triploid range.

Potential and realized reproductive assurance

The potential fruit set, i.e. the number of florets (ovules) per capitulum was fairly the same between the sexual diploid and asexual triploid plants collected in the field (LMM: t = -1.1, p = 0.278), and in the greenhouse (LMM: t = 1.25, p = 0.227; Table 2; Fig. 3A).

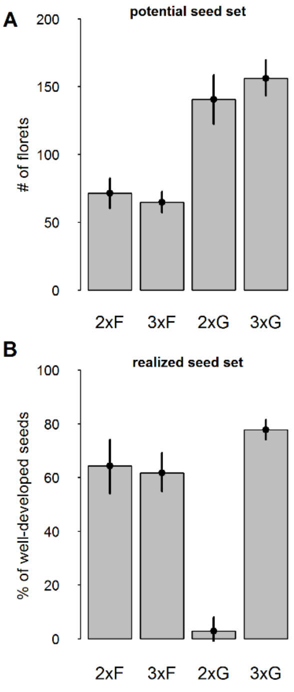

Fig. 3 Barplots of potential, i.e number of florets per capitulum (A) and realized, i.e. proportion of well developed seeds per capitulum, seed sets (B) in strictly sexual diploid (2x) and strictly asexual triploid (3x) plants of *Hieracium alpinum*. F – plants from field sampling, G – plants from the greenhouse – pollination restricted experiment. Bars represent 95% confidence intervals of means derived from mixed effect models using bootstrap approach (10,000 replications).

Whilst the origin of plants had a statistically significant effect on potential fruit set, being two times greater in the plants from the greenhouse than the plants from the field (LMM: χ^2 = 74.99, p < 0.001; Table 2; Fig. 3A), ploidy and the ploidy × origin interaction were statistically insignificant (LMMs: χ^2 = 1. 319, p = 0.330; χ^2 = 0. 949, p = 0.499).

In contrast to our hypothesis, we found no statistical difference in the realized fruit set between sexual diploids and asexual triploids from the field (Table 2; Fig. 3B; LMM: t = -0.546, p = 0.588). However, under the greenhouse conditions with highly restricted access to pollinators, the level of reproductive assurance in sexual diploids dropped down to ca 3 %, while the averaged percentage of well developed fruits per capitulum in asexual triploids reached almost 78% (Table 2; Fig.3B; LMM: t = 22.327, p < 0.001). Both, the mode of reproduction and the origin of plants, and their interaction significantly explained the variation in realized fruit set (LMM: t = -3.416, *p* = 0.003; t = -22.178, *p* < 0.001; t = 19.196, *p* < 0.001).

From four sexual diploid populations repeatedly sampled in two years (2014 and 2015) and which had at least 5 good capitula per year and population, three populations (H073, H074, H077) showed considerable between-years differences in their realized fruit sets (Wilcox non-parametric tests, all *p* < 0.001), being higher in 2015 than 2014 (see Table 1). No between-years difference in this trait was found in the population H057.

The variation in realized fruit sets expressed as coefficient of variation was slightly higher in sexuals than in asexuals under the field conditions (Table 2), and the variance in fruit set was statistically significantly higher in the sexuals (Table 2; Fischer test: F = 1.49, *p* < 0.001). Under the restricted pollination conditions in the greenhouse, the realized fruit set of sexuals varied almost 18-fold more than in asexuals (Table 2).

Though there was no statistically significant association between realized and potential fruit sets in sexual diploid plants from the field(rho = - 0.069, *p* = 0.172), we found significantly negative relationship between these traits in triploid plants collected in natural populations and the greenhouse experiment, respectively (rho = - 0.099, *p* = 0.032; rho = - 0.268, *p* < 0.001, respectively).

Table 2. Summary statistics of potential and realized seed sets of sexual diploid (2x) and apomictic triploid (3x) populations of *Hieracium alpinum* assessed under natural conditions (field) and pollination restricted conditions (greenhouse). Mean values and standard errors were derived from linear mixed effect models. Potential seed set is expressed as the number of florets (= ovules) per capitulum (rounded to entire number). Realized seed set is expressed as percentage of well-developed seeds of all florets per capitulum. CV – coefficient of variation.

variable	**potential seed set (mean ± SE)**		**realized seed set (mean ± SE) [%]**		**variance of realized seed set(CV [%] / variance)**	
ploidy /origin	field	greenhouse	field	greenhouse	field	greenhouse
2x	72 ± 6	141 ± 9	64.3 ± 4.9	2.9 ± 2.9	41.6 / 0.077	366.4 / 0.027
3x	65 ± 4	156 ± 7	61.7 ± 3.7	77.7 ± 1.9	37.7 / 0.051	20.5 / 0.013

Discussion

Flow cytometric screening of a large number of achenes collected across the species range of *H. alpinum* confirmed our assumption about a strict link between ploidy and mode of reproduction in this species. While the diploid progeny arose solely by sexual pathway, the triploid progeny was produced strictly apomictically. We did not find any significant differences between diploids and triploid plants in the capitula size, i.e. in potential fruit set, and this neither in natural populations nor under the greenhouse conditions. Though apomictic plants from natural populations did not show higher fruit sets when compared to sexuals, they varied less in this trait than sexuals suggesting more stable reproductive strategy in the former ones. Furthermore, under conditions of restricted access to pollinators in the greenhouse experiment, apomictic triploids produced in average 25-times more seeds per capitulum than sexual diploids.

Mode of reproduction in H. alpinum *is strictly associated with ploidy level*

Since the first published evidence on parthenogenesis in the genus *Hieracium* s.str. based on the results from castration experiments (Ostenfeld & Raunkiaer 1903), which was subsequently confirmed by cytoembryological observations (Murbeck 1904), apomixis has been considered as obligate or nearly obligate mode of reproduction in *Hieracium* polyploids (Gustafsson 1946; Nogler 1984; Richards 2003; Chrtek et al. 2009). This assumption has been based on the following observations. Firstly, though facultative apomixis in *Hieracium* has been admitted because certain microspecies were still able to produce a various proportion (up to 56 %) of meiotically derived embryo sacs (Bergmann 1935, 1941; Skawińska 1963; Hand et al. 2015), this unusual meiotic behaviour in apomicts invariably led to aborted ovules (Bergmann 1941; Skawińska 1963). Secondly, the fertilization is strongly hampered in polyploid apomicts because of precocious embryony, a phenomenon when the development of an apomictic embryo is usually initiated before flower opening (Bergmann 1935; Skawińska 1963). Finally, polyploid apomicts frequently display disturbed male meiosis leading to low pollen quality and quantity or even complete male sterility what, in turn, significantly diminishes the importance of apomicts as pollen donors (Rosenberg 1917; Gentcheff & Gustafsson 1940; Slade & Rich 2007; Mráz et al. 2009). In contrast to polyploids, diploid *Hieracium* taxa have been considered to be strictly sexual (Gustafsson 1946; Yurukova-Grancharova et al. 2006; Hand et al. 2015) with strong self-incompatibility barrier (Chrtek 1997; Mráz 2003).

Our results based on flow cytometric analyses of well developed achenes confirmed the above mentioned assumptions to full extent. All diploid progeny collected in *H. alpinum*'s diploid range arose by sexual pathway, while those collected in the triploid range arose by apomixis, confirming a strong geographical parthenogenesis pattern in this species as previously suggested by a limited number of karyologically analyzed plants (Mráz et al. 2009 and references therein). Furthermore, our results clearly indicate a very strict association between ploidy and mode of reproduction in *H. alpinum* and confirm the patterns previously assumed based on rare cytoembryological observations (Skawińska 1963), and castration and isolation experiments (Chrtek 1997; Mráz 2003). To our best knowledge, this is the first firm evidence on obligate apomictic formation of functional progeny studied on such large number of progeny and seed plants (2427 achenes from 486 triploid plants were analyzed) collected across considerable part of species range (from the Balkans to the N Scandinavia, i.e. from 43º to 70º of northern latitude). Similarly oriented studies focused on frequency of sexual – asexual reproduction and using flow cytometrical seed screening usually revealed various degree of facultative apomixis in polyploids belonging to various species groups including the genus Boechera (Aliyu et al. 2010), *Hypericum perforatum* (Molins et al. 2014), the *Ranunculus kuepferi* group (Cosendai & Hörandl 2010), the genus *Pilosella* (Krahulcová et al. 2014), *Rubus* subgen. *Rubus* (Šarhanová et al. 2012) or *Taraxacum* sect. *Erythrosperma* (Mártonfiová et al. 2010). Rarely, obligate apomixis has been detected in certain taxa, like in the *Pilosella alpicola* group (Šingliarová et al. 2011), *Rubus* subgen. *Rubus* (Šarhanová et al. 2012), the *Sorbus aria* group (Hajrudinović et al. 2015). However, in all these cases usually a limited number of plants / seeds or fruits per taxon has been analyzed and more intense screening could reveal residual sexuality (Šarhanová et al. 2012).

Level and variability of reproductive assurance in sexuals and asexuals of Hieracium alpinum

Initially, we hypothesized that potential fruit set, i.e. total number of florets (= ovules) per capitulum, will be higher in triploids when compared to diploids due to an increased genome size in the former. Our results based on both, the plants from natural populations and plants cultivated in the greenhouse did not confirm this assumption. Besides the strong positive effect of favorable growing conditions in the greenhouse experiment on potential fruit sets in both cytotypes, the average values of this trait were fairly the same in diploids and triploids within each of the data sets (natural populations *versus* greenhouse experiment). Similar results, i.e. no significant differences between sexual diploid and asexual triploid *Taraxacum* in potential fruit sets were reported by van Dijk (2007). Therefore, our study based on large number of plants originated from large part of the species range do not support a generally accepted view on a significant effect of the whole genome multiplication on plant phenotype (e.g. Levin 1983; Soltis et al. 2007). In contrast, O'Connell & Eckert (2001) found higher number of florets per inflorescence in asexual plants of *Antennaria parlinii* when compared to the sexual ones suggesting higher reproductive effort in the former. Interestingly, the plants of both groups were of the same hexaploid level (O'Connell & Eckert 2001).

Our hypothesis on greater and more stable reproductive assurance of apomictic triploids has been based on the assumption that the pollination environment is unpredictable in time and space (Burd 1994; Ashman et al. 2004; Hamston et al. 2017) and therefore sexual diploids will produce lower and more variable proportion of well developed seeds per capitulum because they fully rely on the presence of compatible mating partners and pollinators mediating pollen transfer. On contrary, asexual triploids are not pollen limited at all, because they do not need compatible partners and insects mediating pollination for successful seed production (Chrtek 1997). Therefore, the realized fruit set of apomictic *H. alpinum* triploids should be higher and less variable than in sexual diploids. In agreement with our hypothesis, the sexual diploids varied significantly more in realized fruit sets than asexual triploids suggesting lower level of predictability / stability in the reproduction output of sexuals.

Our results confirmed also the assumption on higher seed sets in asexuals but only under very restricted greenhouse conditions. In the natural populations the proportion of well developed seeds per capitulum was even slightly higher, though not significantly, in sexual diploids than in asexual triploids of *H. alpinum*. Similar pattern, i.e. no differences in natural populations but strong differentiation under the pollen limited conditions has been observed also in sexual-agamic complex *Antennaria parlinii* (O'Connell & Eckert 1999). In contrast, van Dijk (2007) found that apomictic triploid plants of *Taraxacum* sect. *Ruderalia* had consistently higher realized seed sets than sexual diploid plants growing sympatrically in three populations. We propose several mutually not exclusive explanations for considerable variation in reproductive outputs in natural populations, especially in triploid ones.

As summarized by Stephenson (1981), the overall realized reproductive output of a plant is determined by a number of pollinated and fertilized flowers, level of fruit / seed predation, weather conditions, resource allocation of maternal parent and interactions of all these factors. In our study, we can exclude the impact of fruit predation on the realized fruit sets, as infested samples were excluded from the analyses due to a strong negative effect of fruit predation on realized fruit sets in both, sexual diploids and triploids (see Material and Methods and Hartmann et al. 2017). As stated above, apomictic triploids are fully independent on pollinators and therefore their reproductive output does not depend on presence of compatible partners and presence and activity of pollinators. On the other hand, the sampled diploid plants might not have been limited neither by means of availability of compatible partners (spatial pattern and density of population) nor pollinators. Repeated long-term assessments over several years covering more climatic fluctuations might bring more pronounced differences in seed sets between diploids and triploids. An increase of almost 20% in realized fruit sets of triploid plants cultivated in the greenhouse when compared to the plants from the field (79% *versus* 60%, respectively) rather indicates negative effect of unfavorable weather / habitat conditions for normal seed development, what is a very common factor of seed / fruit abortion in angiosperms (Stephenson 1981; Albert et al. 2001). Furthermore, we found that realized fruit sets in triploid plants seem to be more resource limited than those in diploid plants, as there were significant negative associations between potential and realized fruit sets in the triploids, but not in diploids. In other words, the more ovules were initiated in a capitulum of triploid plants, the more were finally aborted. This pattern was most pronounced under the greenhouse conditions suggesting an increasing competition among ovules (developing fruits) with increasing number of initiating ovules (increasing potential fruit set). Finally, we observed frequent abortion of the 2-3 centermost situated fruits in a capitulum in plants from both, the field and the greenhouse experiments, irrespective of their ploidy level / mode of reproduction. This is a quite common pattern in species which inflorescences develop acropetally (from basal / outer to terminal / inner flowers) and suggests not only differential allocation during inflorescence development but also variation which is inherent in plant axes structure (Diggle 1995).

In addition to external factors, some small portion of aborted seeds could also be due to intrinsic developmental abnormalities of embryo sac / embryo formation. For instance, already Bergman (1935) observed that certain *Hieracium* apomicts aborted high portion of ovules due to failure of embryo sac because of meiosis. Though this and the work by Skawińska (1963) indicated up to 50% of seed abortion likely due to for apomicts an unusual meiotic megasporogenesis, recently published cytoembryological study by Hand et al. (2015) revealed considerably lower rates of meiotic embryo sac formation in apomictic hawkweeds. From 12 studied polyploid accessions belonging to several *Hieracium* species, five did not produce meiotic embryo sacs at all., i.e. they produced solely apomictic diplosporic embryo sacs, while remaining accessions exhibited meiosis in 1-2(-7)% of ovules. This suggests very regular diplosporic embryo sac formation in *Hieracium* polyploids being 100% or very close to this value. Similarly, Noyes & Givens (2013) found very high frequency (in average 95%) of diplosporic embryo sac formation across different tri- and tetraploid *Erigeron annuus* and *strigosus* genotypes cultivated under uniform greenhouse conditions and exhibiting the same type of diplospory (*Antennaria* type) as polyploids of *Hieracium* s.str. Interestingly, despite very high level of diplosporic embryo sac formation, *Erigeron* plants varied a much in their seed sets, being 71% in average (Noyes & Givens 2013). These and our results thus suggest that aberrant meiotic formation in embryo sacs, if occurs, is not the primary factor involved in ovule / seed abortion in apomictic *Hieracium*. Nevertheless, more quantitative data on megasporogenesis / megagametogenesis in *Hieracium* s.str. using cytoembryological clearing technique would be very useful to get more precise picture about putative intrinsic factors associated with embryo sac / embryo abortion.

Conclusions

Obligate apomictic seed reproduction might provide asexuals with superior colonization ability when compared to strictly self-incompatible sexuals because of advantage of uniparental reproduction frequently mirrored in wider geographical ranges of asexuals (Hörandl 2006; Hörandl et al. 2008). Though several recently conducted studies have shown that most of apomicts retain some level of residual sexuality, we found that triploid *Hieracium alpinum* form the seeds by strictly apomictic pathway. To our best knowledge, this is the first firm evidence on obligate apomictic formation of progeny confirmed at large number of plants collected across considerable part of its species range. In contrast, diploid cytotype produced seeds solely by sexual pathway after double fertilization. Contrary to our expectations, the level of reproductive assurance in the natural populations of *H. alpinum* was in average very similar between both cytotypes. We suppose that considerable variability in fruit quality was influenced not only by varying level of pollination in sexuals, but also by environmental conditions, which lowered the level of reproductive assurance in both cytotypes. However, in pollinator-restricted environment asexual triploids produced much higher proportion of well-developed achenes than sexuals did. In addition, the former varied less in reproductive output than sexual diploids. Based on this we hypothesize, that the advantage of more stable and higher reproductive assurance could be important especially during colonization of new habitats, e.g. during postglacial re-colonization when small-sized and fragmented populations were likely arranged in metapopulation-like structures with considerable colonization and extinction dynamics (e.g. Pannel and Barrett 1998; Haag and Ebert 2004). Such specific conditions, which impact also the presence of pollinator and their behavior (Goverde et al. 2002; Albrecht et al. 2010), could considerably lower the colonization / founder success of sexual diploid *H. alpinum*, which strictly relies on availability of mating partners and pollinators (Chrtek 1997; Mráz 2003). Whether this mechanism was also the main factor for the replacement of diploid sexuals by triploid asexuals in the current range of triploids remains, however, unclear. Ideally, the differences in medium / long term fitness between sexuals and asexuals, should be tested in in-situ mixed-ploidy experiment. However, because both cytotypes currently occur in strict allopatry, the reciprocal transplant experiment cannot be conducted due to an ethical issue. Alternatively, experimentally created ex-situ mixed cytotype populations at lowland conditions, under which *H. alpinum* performs very well (Mráz et al. unpubl.), could be a promising approach to get a new insight on the fate of both cytotypes.

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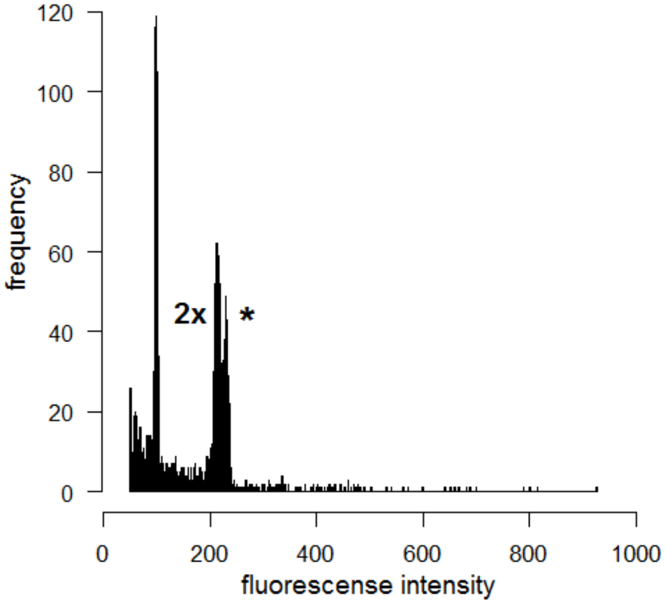
Supporting Information

Fig. S1 Flow cytometric analysis of diploid (“2x”, H57_11_1) and putatively hyperdiploid seedlings (“*”, H57_11_2) of *Hieracium alpinum*. The leftmost peak corresponds to a standard plant *Bellis perennis*.



PAPER IV

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Seed traits, terminal velocity and germination in sexual diploid and apomictic triploid *Hieracium alpinum* (Asteraceae): Are apomicts better dispersers?
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Photo: *Hieracium alpinum* with ripe achenes shortly before dispersal (Photo credit: P. Mráz; Furkapass, 2015)

Seed traits, terminal velocity and germination in sexual diploid and apomictic triploid *Hieracium alpinum* (Asteraceae): Are apomicts better dispersers?

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Abstract

In this study, we examined the effect of seed characteristics (seed weight, seed length, seed width, pappus length and pappus width) and ploidy level on seed terminal velocity and germination in *Hieracium alpinum*, an arcto-alpine anemochorous species with vicariant diploid sexual and triploid apomictic populations producing diploid and triploid seeds, respectively. Importantly, triploids occupy a considerably larger range than diploids, suggesting that they have better colonization abilities putatively mediated by increased dispersal potential of the former. In total, 970 seeds collected from 228 mother plants from 30 populations across the distribution range were analysed. Diploid and triploid seeds did not differ significantly in most morphological traits, except for triploid seeds being wider. Terminal velocity was greater in diploid seeds compared to triploid seeds. This difference was not significant if a nested design (ploidy, population, mother plant) was used but significant if the population level was not considered, indicating high interpopulation variation in this trait. Terminal velocity was positively and significantly correlated with seed mass, length and width and negatively correlated with pappus length and width. Germination probability was positively associated with seed mass and negatively with ploidy (higher in diploids, compared to triploids). Our results thus indicate partly opposite dispersal-establishment strategies of diploid sexual and triploid apomictic biotypes of *H. alpinum*. One the one hand, the slightly greater values of terminal velocity of diploid seeds might reduce their dispersal potential, but on the other, their significantly greater germination rates, when compared to triploid seeds are likely to allow their more successful establishment. To sum up, our result do not support the hypothesis of heavier seeds with higher terminal velocity and higher germination rates in autopolyploids compared to their diploid progenitors.

Keywords: Geographical parthenogenesis, Germination, Polyploidy, Postglacial migrations, Seed dispersal

Introduction

Geographical parthenogenesis describes the phenomenon when closely related or even conspecific sexual and asexual organisms have different distribution patterns. Compared to sexuals, asexuals are distributed more broadly and tend to occur in higher latitudes and altitudes (Bierzychudek, 1985; Van Dijk, 2003; Kearney, 2005; Hörandl, 2006,2009; Cosendai et al., 2013). Several hypotheses have been put forward to explain the causality of geographic parthenogenesis, but none of them alone seems to be unequivocal (Hörandl, 2006). First, Baker's Law (Baker, 1965, 1967) supposes that asexual plants are better colonizers, as they are theoretically able to establish a new population with one individual only, which markedly increases the likelihood of successful long-distance dispersal whereas their sexual relatives are often dependent on mating partners. The hypothesis that polyploidy and/or a hybrid origin brings an advantage assumes a strong association between polyploidy and apomixis (asexual seed reproduction, Asker and Jerling, 1992). The distributional success of asexual plants can also be explained by whole genome duplication providing the asexuals with higher levels of genetic variation and heterozygosity, which might increase their ecological tolerance and buffer the negative effect of deleterious mutations (Bierzychudek, 1985; Asker and Jerling, 1992). Moreover, polyploidy might alter phenotypic traits, including those involved in dispersal, so differences in ploidy level could tentatively explain differences in colonization ability between diploids and polyploids (Linder and Barker, 2014). Further hypotheses concern more efficient exploitation of ecological niches by asexuals due to either superior specialization (Frozen niche variation hypothesis; Vrijenhoek, 1984; Vrijenhoek and Parker, 2009) or superior generalization (General-purpose genotype hypothesis; Parker et al., 1977), lack of inbreeding depression during colonization/recolonization events in asexuals (Metapopulation hypothesis; Bierzychudek, 1985; Haag and Ebert, 2004). Finally, an evolutionary advantage of sexuals in environments rich in biological interactions (competitors, parasites and pests) is supposed, connected with a greater frequency of asexuals in marginal and harsh habitats in northern latitudes, where biotic interactions are less important (Asker and Jerling, 1992; Red Queen hypothesis − Van Valen, 1973).

Colonization success in plants is generally affected by numerous morphological and physiological traits, among others by seed size, velocity and germination, which have either positive or negative effects on the two early key-steps in colonization − dispersal and establishment. Whereas seed dispersal in wind-dispersed species seems to be negatively correlated with seed mass by increasing the seed settling velocity (Greene and Johnson, 1993; Greene and Quesada, 2005), seed germination and seedling growth is positively linked to increasing seed mass, regardless of the mode of dispersal (Westoby et al., 1996; Turnbull et al., 1999; Turnbull et al., 2004). Wind-dispersed plants may thus trade dispersal success for germination and establishment success by increasing seed mass (Strykstra et al., 1998; Soons and Heil, 2002; Debain et al., 2003; Jakobsson and Eriksson, 2003; Skarpaas et al., 2011).

Seeds may therefore be a result of complex adaptive compromises, because different seed characteristics are required for different functions. Seed traits, especially weight, within species were considered for a long time to be relatively constant (Harper et al., 1970). More recently, intraspecific variation has been reported in many species; it can be attributed to many sources (e.g. Susko and Lowett-Doust, 2000; Castro et al., 2006; Cochrane et al., 2014), including autopolyploidization (Bretagnolle et al., 1995). Autopolyploidization is known to alter plant morphology and physiology, and consequently mediate a shift in ecology (Paterson, 2005; Ramsey, 2011) that could predispose polyploid cytotype(s) to being better colonizers. However, a detailed comparison of seed morphology, velocity and germination in sexual diploid and apomictic polyploid cytotypes within one species is still lacking. In this study, we explore the role of polyploidy in shaping seed traits in the arcto-alpine species *Hieracium alpinum*. This species possesses several unique features which make it suitable model for such a study. First of all, it perfectly exemplifies the pattern of geographical parthenogenesis (Mráz et al., 2009). Secondly, apomictic triploids are autopolyploids, originating from sexual diploids (evidence from molecular markers, Mráz et al., unpubl.). Specifically, we attempted to answer following questions: (i) What are the differences between sexual diploids and apomictic triploids with respect to seed morphology and seed mass? (ii) What are the effects of particular seed characteristics and ploidy on seed velocity and seed germination? (iii) Can the colonization success of agamospermous triploids be explained by their dispersal biology? To answer these questions we searched for intercytotype differences in six seed traits (length and width of seeds, length and width of pappus, terminal velocity and seed mass) and the germination rate measured on 970 seeds collected from 228 mother plants originated from 30 natural populations.

Material and methods

Species

Hieracium alpinum is an herbaceous, arcto-alpine species with a distribution area extending from Greenland, through Iceland, the mountains of Britain and Scandinavia, to north-western Russia. The distribution area in Central Europe includes the Alps, the Sudeten Mts and further eastwards the Carpathians. Some isolated exclaves occur in the Vosges and Harz Mts and on the Vranica Plateau (Bosnia and Herzegovina); records exist also from the Apennino Centrale and the Apuane Mts in Italy (Bräutigam, 1992). *Hieracium alpinum* comprises two geographically vicariant cytotypes with different modes of reproduction. Whereas diploids are confined to the Eastern and Southern Carpathians and are strictly sexual and self-incompatible (sporophytic self-incompatibility), triploids occur in the rest of the species' geographic range (Mráz et al., 2009) and are agamospermous. The female meiosis in triploids is omitted, they produce mitotically derived unreduced embryo sac (diplospory of the Antennaria type; Skawińska, 1963). The egg cell develops parthenogenetically, the central cell of the embryo sac does not need to fuse with the spermatic cell (endosperm develops autonomously). Both cytotypes differ also in their male function. While diploids produce a lot of homogenously sized pollen, triploids are usually pollen sterile, only rarely form a small amount of heterogeneously sized pollen (Chrtek Jun., 1997; Slade and Rich 2007; Mráz et al., 2009). The seeds of *H. alpinum* possess well developed pappus and are dispersed by wind, although dispersal by animals (exozoochory) cannot be fully excluded.

Sampling

Seeds were collected in the field in Scandinavia, in the Alps, the Sudeten Mts, the Carpathians and on the Vranica Plateau in Bosnia and Herzegovina, covering representatively the distribution range of diploids and nearly the range of triploids. As a rule, five well-developed seeds per each of usually 5–12 mother plants per locality were used for measurements, yielding a total number of 970 seeds (Suppl. Table 1). After collection, the seeds were stored at room temperature for c. 4–6 weeks, after which they were placed into a refrigerator with the temperature set to c. 4 °C.

Seed traits and germination

In total, 270 seeds from 63 diploid plants originating from 7 populations and 700 seeds from 165 triploid plants originating from 23 populations were measured and included in our germination experiment. Five seed traits, assumed proxies of seed velocity and germination, were measured on the level of individual seeds, i.e. every seed was measured separately. Seed length (without pappus) and width (without pappus), pappus length and width were measured using ImageJ 1.49 software (Schneider et al., 2012) from photos taken under a stereomicroscope, and seed mass was ascertained using an ultra microbalance Metler Toledo with the precision of 0.0001 mg. Seed velocity was measured using the method of Askew et al. (1997). The apparatus determines the rate of fall of seeds in air by detecting their passage through two fans of laser light. After the measuring of these traits, the seeds were placed on moistened filter paper on glass beads in water-filled Petri dishes randomly placed in growth chambers (Thermoforma 3, Model 3744, Thermo Fisher) with a cool daylight light source (Colourlux plus, LT 15 W/840 G13, NARVA) at 12 h photoperiod at 20/10 °C. The germination was recorded daily over a period of 51 days, whereby, to ensure randomness, the horizontal and vertical positions for all Petri dishes inside the germination chambers were daily shifted. Emerged seedlings were removed and individually placed on planting trays until ploidy level analyses.

Table 1. Mean values and SD of seed traits in diploid and triploid *Hieracium alpinum* and results of LMMs testing the effect of ploidy on seed traits (for the effect of ploidy on velocity see Table 2). Seed family and population were used as random terms. Seed length refers to length without the pappus.

Seed trait/ploidy level	**2x (mean ± SD)**	**3x (mean ± SD)**	**χ^2**	***p***
Seed mass (mg)	0.862 ± 0.209	0.829 ± 0.204	0.232	0.63
Seed length (mm)	3.652 ± 0.387	3.671 ± 0.407	0.019	0.891
Seed width (mm)	0.688 ± 0.063	0.744 ± 0.073	7.489	**0.006****
Pappus length (mm)	5.945 ± 0.830	6.339 ± 0.766	2.609	0.106
Pappus width (mm)	6.120 ± 1.510	6.313 ± 1.289	0.11	0.74
Velocity ($m.s^{-1}$)	0.741 ± 0.127	0.672 ± 0.137	1.926	0.165

Flow cytometric seed screening

At the end of the experiment we ascertained the ploidy level of every non-germinated but viable seed or emerged seedling in planting tray using flow cytometry to asses their ploidy level and, in the case of not-germinated seeds, also the mode of reproduction (sexual *versus* apomictic). The ploidy level of each sample was inferred as a relative position of the peak corresponding to the nuclei of the sample (either cell nuclei from leaf tissues in the case of seedlings or cell nuclei of embryos in the case of seeds) to the peak corresponding to the internal standard (see below). In addition, using flow cytometric seed screening analyses, we determined whether seeds arose via the sexual or the apomictic pathway. This discrimination is very straightforward and is based on different ploidy ratios between the embryo and the endosperm (Matzk et al., 2000). In seeds formed via the sexual pathway then the ratio is always 1.5 because of double fertilization (i.e. in diploid *H. alpinum* the embryo is diploid and the endosperm is triploid), whereas in apomictically derived seeds the ratio always equals 2 (in triploid *H. alpinum*, the embryo is triploid and the endosperm is hexaploid) because of a mitotically produced embryo sac (see above). The samples were analysed using a two-step procedure following Otto (1990) and Doležel and Göhde (1995) with simultaneously chopped leaves of *Bellis perennis* as the internal standard and 4,6-diamino-2-phenylindole (DAPI) as the fluorescent stain on a Partec Cyflow instrument equipped with an HBO lamp. The ploidy level of embryos and the endosperms was determined as the relative position of the peaks corresponding to either of the tissues to the peak of the internal standard.

Data analysis

First, the effect of ploidy on particular morphological seed characteristics (seed mass, seed length, seed width, pappus length and pappus width) was tested using linear mixed effect models (LMM) with seed family nested within population, both treated as random factors.

Secondly, in order to explain the effects of particular seed characteristics (seed mass, seed length, seed width, pappus length and pappus width) and ploidy level (2 x *versus* 3x) on (i) terminal velocity and (ii) germination, we used linear mixed effect models (LMM; seed velocity) and generalized linear mixed effect models with a logit link function for binomial data (GLMM; seed germination), respectively. Seed family nested within population and ploidy (except for testing for the effect of ploidy, where only the seed family was nested within population) were treated as random factors. The analyses were performed using the 'lmer' and 'glmer' functions implemented in the lme4 package (Bates et al., 2015) in R (R Core Team, 2013), and the statistical significance of terms was assessed using the likelihood ratio test (LRT) comparing a fully fitted model to the model from which the tested term was removed.

Finally, we constructed a multiple linear regression model with all seed variables (correlation coefficient values between all pairs of variables did not exceed 0.5, so all traits were included) and their interactions to find the best predictors of (a) terminal velocity and (b) germination rate. We used the 'glmulti' function from the 'glmulti' library (Calgano and de Mazancourt, 2010) and information criteria (AIC, BIC) to select the best model.

Results

Morphological seed traits

Diploid and triploid seeds differed in none of the traits measured except seed width (Table 1, Fig. 1). Triploid seeds were significantly wider than diploid seeds.

Terminal velocity

Terminal velocity was slightly greater in diploid seeds (0.741 ms^{-1} ± 0.127) compared to triploid seeds (0.673 ms^{-1} ± 0.137; mean ± SD; Fig. 1F). This difference was not significant if a nested design (populations, plants) was used (χ^2=1.926, p=0.165) but significant if the population level was not considered (χ^2=14.767, $p < 0.001$), indicating high interpopulation variation in this trait. When testing seed traits separately, terminal velocity was positively and significantly correlated with seed mass, seed length and seed width, and negatively correlated with pappus length and pappus width (Table 2). When considering multiple predictors of terminal velocity and their possible interactions, the best linear model explained almost 63% of the variation in terminal velocity, with seed mass (Fig. 2), pappus length and pappus width as the best predictors (Table 3).

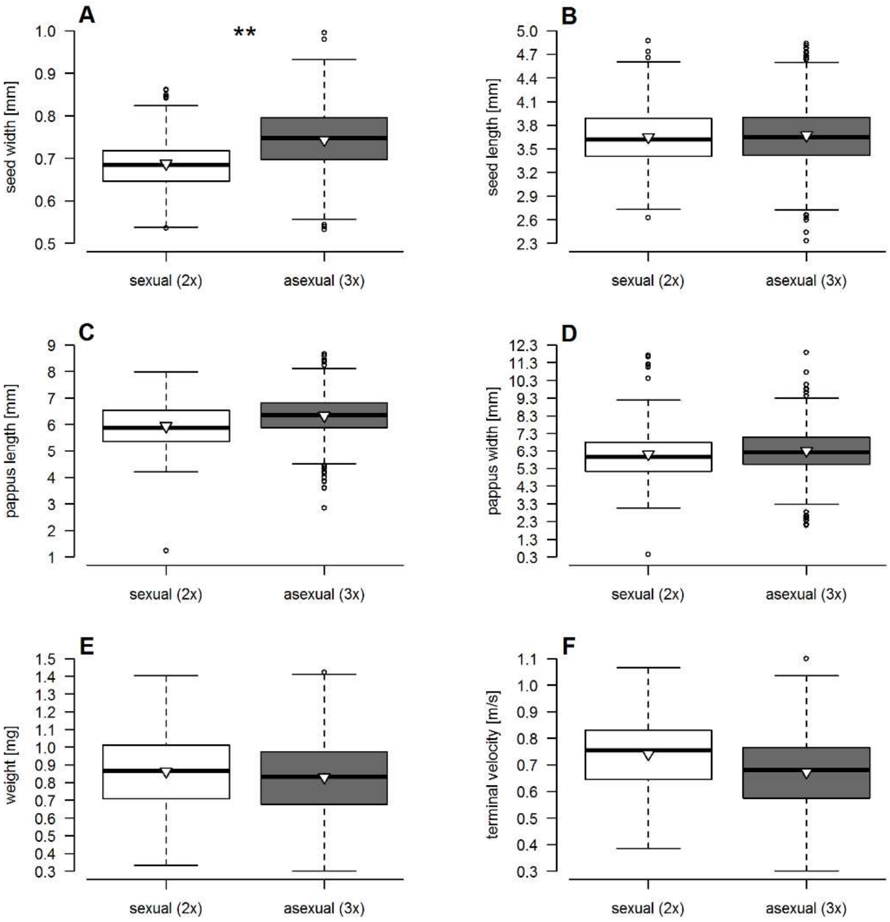

Fig. 1. Variance of selected traits in diploid and triploids seeds of *Hieracium alpinum*. Medians, 25–75th percentile range, non-outlier ranges and outliers are presented; n(diploids)=270, n(triploids)=700.

Table 2. Results of LMMs testing separately the effect of seed traits and ploidy level on seed velocity in diploid and triploid *Hieracium alpinum*. Presented are χ^2 values and corresponding p values obtained from LRT tests. Seed family nested within population and (except for testing the effect of ploidy) ploidy were used as random terms. Directions of arrows indicate either a positive (↑) or a negative (↓) relationship.

Seed trait	χ^2	*p*
Seed mass	426.78	<0.001***↑
Seed length	34.797	<0.001***↑
Seed width	16.298	<0.001***↑
Pappus length	64.603	<0.001***↓
Pappus width	103.54	<0.001***↓
Ploidy (2x, 3x)	1.926	0.165

Table 3. Results of linear model based on the best predictors of seed velocity and their combinations in diploid and triploid *Hieracium alpinum*.

Seed trait	Estimate (slope)	*p*
Seed mass	0.876	<0.001 ***
Pappus length	−0.121	<0.001 ***
Pappus width	−0.173	<0.001 ***
Seed mass×seed length	−0.264	<0.001 ***
Pappus length×seed width	0.117	<0.001 ***
Pappus width×seed length	0.028	<0.001 ***
Pappus length×pappus width	−0.005	0.002 *

Seed germination

The probability of germination was significantly greater in diploid (81.9%) than in triploid (65.3%) seeds. When testing the predictors separately, the probability that a particular seed germinated within 51 days was significantly positively associated with seed mass and negatively with ploidy (Table 4). When considering multiple predictors of germination and their interactions, the best predictors were seed mass and its interaction with seed width and seed length (Table 5).

Table 4. Results of GLMMs testing separately the effect of seed traits and ploidy on germination of seeds (12 h of daylight/12 h of darkness at 20/10 °C with a cool daylight light source for 51 days) in diploid and triploid *Hieracium alpinum*. Presented are χ^2 values and corresponding p values obtained from LRT tests. Seed family nested within population and (except for testing the effect of ploidy) ploidy were used as random terms. Directions of arrows indicate either a positive (↑) or a negative (↓) effect of seed traits on the rate of germination.

Seed trait	χ^2	*p*
Seed mass	43.123	**<0.001***↑**
Seed length	0.057	0.811
Seed width	2.22	0.136
Pappus length	0.007	0.931
Pappus width	0.423	0.515
Ploidy (2x, 3x)	11.005	**<0.001***↓**

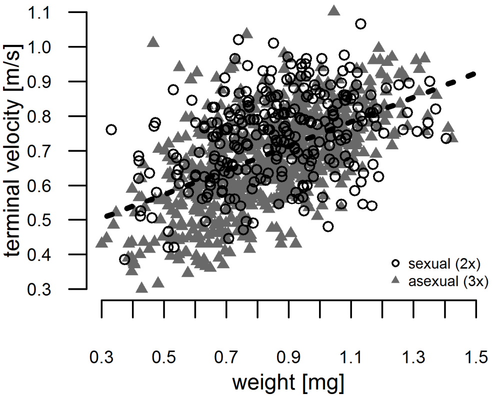

Fig. 2. Relation between terminal velocity and seed mass (weight) of *Hieracium alpinum* diploids and triploids.

Table 5. Results of a generalized linear model based on the best predictors of seed germination and their combinations in diploid and triploid *Hieracium alpinum*. Ploidy level was not considered.

Seed trait	Estimate (slope)	*p*
Seed mass	10.401	**<0.001*****
Seed widthxseed mass	5.438	**<0.001*****
Seed length	-0.622	**0.001****

Discussion

In the present study we found significant differences in none but one (seed width) of the morphological seed traits between sexual diploids and agamospermous triploids of *Hieracium alpinum*. We also revealed partly opposite dispersal-establishment strategies of sexual diploids and apomictic triploids, the former having slightly but non-significantly greater values of terminal velocity (but significantly if the effect of population was excluded), i.e. likely lower dispersal potential but greater germination rates. Variation in terminal velocity can mostly be attributed to seed mass (weight), pappus length, pappus width and interaction between pappus length and seed mass. By contrast, germination success depended nearly exclusively on the seed mass. We cannot distinguish between the effects of a whole genome duplication (polyploidy) and of a shift to apomixis as both factors are tightly linked in *H. alpinum*. However, while it is well known that genome duplication can alter phenotypic traits, the effect of the shift to apomixis is most likely much weaker.

Seed traits, terminal velocity and germination in diploid and triploid cytotypes

Our results are not in line with the general trend that polyploids have heavier seeds than conspecific or closely related diploids. Although heavier seeds in natural tetraploids compared to diploids were reported in *Dactylis glomerata* (Maceira et al., 1993) and *Vicia cracca* (Eliášová and Münzbergová, 2014), Hahn et al. (2013) did not find any difference between European diploid and tetraploid populations of *Centaurea stoebe* in any of the measured seed traits, including seed mass; however, pappus length was greater in diploid populations. Greater values of seed mass were also found in synthetic autotetraploids of *Hylocereus monacanthus* (Cohen et al., 2013), *Catharanthus roseus* (Hosseini et al., 2013) and *Lavandula angustifolia* (Urwin et al., 2007). Differences in seed size or mass might be also attributed to factors other than ploidy. Several studies have noted architectural and physiological constraints (McGinley et al., 1987; Diggle, 1995), clinal pattern of seed mass variation with respect to both altitude (Lord, 1994) and latitude (Baker, 1972; Hurka and Benneweg, 1979), and declining seed mass over the course of the reproductive season (Susko and Lowett-Doust 2000; Baskin and Baskin 2014).

We confirmed the generally accepted positive correlation between seed mass and terminal velocity (Greene and Johnson, 1993; Greene and Quesada, 2005; Hahn et al., 2013). Our value of terminal velocity for *H. alpinum* (0.690 ms^{-1} ± 0.138; mean ± SD) was lower in compared to the single value listed for this species in the LEDA database (0.929 ms^{-1}; Lutz in Kleyer et al., 2008). However, the exact method used for the ascertainment of the terminal velocity of this accession is not provided, so a serious comparison is not possible. The mean terminal velocity for the rest of the 77 LEDA entries referring to 11 species of *Hieracium* s. str. (*Hieracium* subgen. *Hieracium*, data referring to species nowadays separated into the genus *Pilosella* were excluded) is 0.775 ms^{-1} ± 0.194; mean ± SD), which more or less corresponds to our data. We also detected high interpopulation variation in terminal velocity, which strongly affected the differences between ploidy levels (terminal velocity between ploidies significantly differed if the population level was not considered, otherwise the difference was not significant). The variation in this trait mediated by variation in seed mass and pappus traits might be caused by environmental factors, such as nutrient supply, climatic conditions or competition as well as genetic factors (Wulff, 1986; Halpern, 2005; Völler et al., 2012; Kołodziejek, 2017).

Seed germination is, as a rule, positively linked to increasing seed mass, regardless of the mode of dispersal (Westoby et al., 1996; Turnbull et al., 1999; Turnbull et al., 2004), which well coincides with our data for *H. alpinum*. By contrast, the evidence of an impact of genome duplication on germination in autopolyploid complexes is inconsistent. Bretagnolle et al. (1995) demonstrated that ploidy has a direct impact on seedling emergence irrespective of seed size − seeds from tetraploid plants of *Dactylis glomerata* germinated faster and in a greater percentage than those of equal weight from diploid plants. A similar relation was confirmed by Eliášová and Münzbergová (2014) in diploid and tetraploid cytotypes of *Vicia cracca* and Haouala et al. (2009) in diploids and colchicine-induced tetraploids of *Trigonella maritima*. On the other hand, it has been argued that a reduction in germination rate after autopolyploidization may occur in *Hylocereus monacanthus* and *Catharanthus roseus* (Cohen et al., 2013; Hosseini et al., 2013). No effect of ploidy on the rate of germination was recorded, for example, in *Chamerion angustifolium* (Burton and Husband, 2000) or *Centaurea stoebe* (Hahn et al., 2013). The interdependence of seed size and ploidy on seed germination is thus very complex and requires further investigation.

Are triploid apomicts better colonizers due to better seed dispersal?

Compared to sexually derived seeds, lighter and thus better dispersible apomictically derived seeds might possess an advantage in colonizing new areas following glacial retreat (Hui and Richardson, 2017). However, this statement must be treated with caution and in the frame of other putatively driving factors of geographical parthenogenesis. First, the reproductive independence of apomicts seems to be the principal advantage especially in harsh environments and in longdistance dispersal. A larger seed set as a result of reproductive assurance can be expected in apomicts compared to sexual diploids; however, this was not confirmed in *H. alpinum* (Mráz et al., unpubl.). A different extent or intensity of herbivory or pathogen attack can also affect the fitness of diploid and polyploid plants (Nuismer and Thompson, 2001; Münzbergová, 2006; Arvanitis et al., 2010). Hartmann et al. (2017) examined seed predation in diploid and tetraploid populations of *H. alpinum*, but found no significant differences in either its frequency or intensity. The range expansion of polyploid apomicts may also be related to the increased ecological tolerance of polyploids given by their genomic constitution, reproductive assurance by apomixis being only a supportive factor (Schinkel et al., 2016). As regards germination, lower germination rates found for triploid seeds might be partly caused by increased seed dormancy detected in some triploid populations (Hartmann et al., unpubl.). Finally, seed dispersal can be shaped by factors other than velocity, such as release height and environment (especially vegetation). However, recent diploids and triploids do not differ in stem high and vegetation structure is the same for both cytotypes (Hartmann et al., 2017).

Conclusions

In this study we observed partly opposite dispersal-establishment strategies of geographically vicariant sexual and agamospermous biotypes of *H. alpinum*. Diploid seeds have slightly but not significantly greater values of velocity and significantly greater germination rates compared to triploid seeds. Lower seed mass and lower velocity of triploid seeds thus might have contribute to an expansion of triploid cytotype in the postglacial period. The observed variation in the velocity can be explained by seed mass, pappus length, pappus width and the interaction between pappus length and seed mass; variation in germination depends nearly exclusively on the seed mass.

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Supporting Information

Table S1. – Details of the localities, including geographical coordinates, altitude, date and numbers of mother plants and seeds. AP – the Alps, DM – Dinaric Mountains, EC – Eastern Carpathians, SC – Southern Carpathians, SU – Sudeten Mts, SV – Scandinavia, WC – Western Carpathians.

Population	**Locality**	**N**	**E**	**Altitude (m)**	**Collecting date**	**Plants**	**Seeds**
sexual diploid populations							
C1	RO, Rodnei Mts, the Şaua Gargalău saddle (EC)	47.58	24.80	1770	12-Aug-14	10	47
H57	RO, Bistriţa Mts, Mt. Pietrosul Bogolin (EC)	47.39	25.54	1693	11-Aug-14, 30-Aug-15	12	42
H73	RO, Parăng Mts, Mt. Parăngul Mic (SC)	45.38	23.51	2099	07-Aug-14, 13-Aug-15	10	43
H74	RO, Buçegi, Mts, Mt. Babele (SC)	45.41	25.48	2120	09-Aug-14, 15-Aug-15	5	19
H77	RO, Fagaraş Mts, Mt. Arpaşu Mic (SC)	45.59	24.63	1944	08-Aug-14, 14-Aug-15	10	43
H63	UA, Svydovets Mts, Mt. Stih (EC)	48.25	24.23	1416	28-Jul-14	10	46
H67	UA, Skchidni Beschady Mts, Mt. Pikuj (EC)	48.86	23.00	1357	21-Jul-14	6	30
apomictic triploid populations							
H20	AT, Defereggengebirge, Mooseralm, (AP)	46.89	12.37	2359	22-Aug-14	8	34
H26	AT, Schladminger/Wölzer Tauern, Sölkpass, (AP)	47.27	14.08	1692	21-Aug-14	3	15
H87	BiH, Vranica planina Mts, Mt. Nadkrstac, (DM)	43.96	17.74	2040	05-Aug-14	10	41
H3	CH, Wallis, Simplonpass, (AP)	46.26	8.01	2300	13-Aug-15	4	19
H4	CH, Wallis, Furkapass, (AP)	47.73	14.27	2128	23-Aug-14	1	4
H82	CZ, Krkonoše Mts, Mt. Studniční hora, (SU)	50.73	15.71	1416	16-Aug-14	7	31
H110	FR, Massif de la Vanoise, Les Evettes, (AP)	45.37	7.11	2599	24-Aug-14, 28-May-15	9	43
H111	FR, Massif du Beaufortain, Col du Cormet de Roselend, (AP)	45.69	6.70	2137	24-Aug-14	9	40
H100	NO, Nordland, Arctic Polar Circle, (SV)	66.55	15.33	729	26-Aug-14	10	37
H101	NO, Nordland, Narvik, Gáicajávrrit (SV)	68.46	18.03	461	27-Aug-14	2	5
H102	NO, Storfjord (SV)	69.05	20.74	561	27-Aug-14	9	34

Table S1 continued

H103	NO, Finmark, Bigás, (SV)	70.16	23.75	505	28-Aug-14	10	41
H94	NO, Telemark, bellow Mt. Gaustakane, (SV)	59.82	8.73	1270	21-Aug-14	10	41
H95	NO, Øystre Slidre, Mt. Båtskardet stølane, (SV)	61.31	8.81	1097	22-Aug-14	6	26
H99	NO, Mo i Rana, Mt. Hellerfjellet, (SV)	66.2	14.5	871	25-Aug-14	10	42
S2A	NO, Nordland, Steinfjellet, (SV)	64.87	13.26	703	24-Aug-14	3	10
S2B	NO, Nordland, Steinfjellet, (SV)	64.87	13.27	737	24-Aug-14	2	8
S3A	NO, Nordland, Steinfjellet, Mt. Bjørkvassklumpen, (SV)	64.87	13.28	733	24-Aug-14	9	36
S3B	NO, Nordland, nearby the Steinfjellet-tunnel , (SV)	64.86	13.25	596	24-Aug-14	9	35
H96	NO, Oppland, Vang al Vaji (SV)	62.45	9.68	1220	23-Aug-14	4	17
H33	SK, Nízke Tatry Mts, Mt. Chopok, (WC)	48.94	19.32	1961	10-Aug-14	10	48
H42	SK, Nízke Tatry Mts, Mt. Kráľova hoľa, (WC)	48.89	20.13	1931	09-Aug-14	11	51
H47	SK, Vysoké Tatry Mts, Mt. Veľká Svišťovka, (WC)	49.20	20.24	1936	13-Aug-14, 28-Aug-15	9	42

APPENDIX

Curriculum Vitae – Matthias Hartmann

* November, 30th, 1987; Grevesmühlen, Germany

EDUCATION & EMPLOYMENT HISTORY

since 06/2018	Institute of Botany of the Czech Academy of Sciences, Průhonice, Czech Republic PhD studies
since 10/2014	Charles University Prague, Department of Botany, Faculty of Science PhD studies PhD thesis: “Geographical parthenogenesis: evolutionary and ecological significance of apomictic reproduction in vascular plants” (supervisor: Patrik Mráz)
04/2014 – 12/2016	Charles University, Department of Botany, Faculty of Science Research Associate
10/2011 – 03/2014	Martin-Luther-University Halle-Wittenberg Studies Masters of Biology Chosen focuses: Immunology and human genetics Conservation ecology (heathland management) Population- and field-ecology Master thesis: “The role of genetic admixture for a successful invasion: a case study with *Centaurea stoebe* s.l. L.” Mark for thesis: 1.3 (excellent) Final mark: 1.4 (excellent)
10/2008 – 09/2011	Martin-Luther-University Halle-Wittenberg Studies Bachelor of Biology

Bachelor thesis: „Comparison of the genetic structure and diversity of native and invasive *Ulmus pumila* populations"
Mark for thesis: 1.2 (excellent)
Final mark: 2.1 (good)

PROJECT / SCIENTIFIC EXPERIENCE

10/2013 Martin-Luther-University Halle-Wittenberg
Employment as student assistant researcher

Supervising undergraduate course "habitat- and population ecology"

06/2013 – 07/2013 Senckenberg Museum of Natural History Görlitz
Employment as student assistant researcher

Analyses of soil samples

10/2012 Martin-Luther-University Halle-Wittenberg
Employment as student assistant researcher
Supervising undergraduate course "habitat- and population ecology"

02/2012 – 03/2012 two months internship at the University of Wisconsin Madison; Department of Horticulture; working group of Prof. J.E. Zalapa

Extraction of *Ulmus minor* – DNA and scoring microsatellites profile

05/2011 Martin-Luther-University Halle-Wittenberg
Employment as student assistant researcher

DNA-Extraction ATMAB-Extraction

PUBLICATIONS (published and submitted)

2018 Mráz, P.; Zdvořák, P.; **Hartmann, M**.; Štefánek, M.; Chrtek, J.
Can obligate apomixis and more stable reproductive assurance explain the distributional successes of asexual triploids in Hieracium alpinum *(Asteraceae)?*
Plant Biology (resubmitted)

2018	**Hartmann, M**.; Jandová, K.; Chrtek, J.; Štefánek, M.; Mráz, P. *Effects of latitudinal and elevational gradients exceed the effects of between-cytotype differences in eco-physiological leaf traits in diploid and triploid Hieracium alpinum* Alpine Botany (resubmitted)
2018	Chrtek, J.; **Hartmann, M**.; Mrázová, V.; Zdvořák, P.; Štefánek, M.; Mráz, P. *Seed traits, terminal velocity and germination in sexual diploid and apomictic triploid* Hieracium alpinum *(Asteraceae): Are apomicts better dispersers?* Flora 240:76 — 81
2017	**Hartmann, M**.; Štefánek, M.; Zdvořák P.; Heřman P.; Chrtek J.; Mráz P. *The Red Queen hypothesis and geographical parthenogenesis in the alpine hawkweed* Hieracium alpinum *(Asteraceae*) Biological Journal of the Linnean Society 122:681 — 696
2017	Rosche, C.; Hensen, I.; Mráz, P.; Durka, W.; **Hartmann, M.**; Lachmuth, S. Invasion success of polyploids: The role of self-incompatibility and inbreeding depression for the contrasting colonization ability of diploid and tetraploid *Centaurea stoebe* s.l. Journal of Ecology 105:425 — 435
2017	Hirsch, H.; Brunet, J.; von Wehrden, H.; **Hartmann, M.**; Kleindienst, C.;Schlautman, B.; Kosman, E.; Wesche, K.; Renison, D., Hensen,I. *Intra- and interspecific hybridization in invasive Siberian elm* Biological invasions 19:1889 — 1904
2016	Durka, W.; Hensen, I.; Mráz, P.; **Hartmann, M**.; Müller - Schärer, H.; Lachmuth, S. *The population genetics of the fundamental cytotype-shift in invasive* Centaurea stoebe *s.l.: genetic diversity, genetic differentiation and small-scale genetic structure differ between cytotypes but not between ranges* Biological Invasions 18:1895 — 1910
2016	Hirsch, H.; Hensen, I.; Wesche, K.; Renison, D.; Wypior, C. Kleindienst, C.; **Hartmann, M.**; von Wehrden, H.

Non-native populations of an invasive tree outperform their native conspecifics
AoB PLANTS 8: plw071

EXTRACURRICULAR ACTIVITIES

since 11/2017	German teacher in language school “StudioKlar”