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# Autopolyploids: particularly hopeful monsters

# Autopolyploidi: obzvláště slibné hříčky přírody

BACHELOR THESIS

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# ABSTRACT

Autopolyploidy, genome duplication *per se*, is a severe mutation which presents both great challenge and great opportunity for the species which has undergone it. First, a whole series of initial challenges has to be overcome, e.g., establishment within diploid parental population, proper functioning of the cell with doubled genetic information and restoration of proper mitosis and meiosis. The population genetic changes can become beneficial afterwards as the two times higher effective population size and polysomic inheritance increase heterozygosity and genetic variability within the new polyploid lineage. It also reduces negative impacts of genetic drift and inbreeding depression. In evolutionary context, having two genomes allows selection to be more relaxed, thus genes can quickly diversify into alleles with new function or sub-function. To better understand the molecular mechanisms of selection on a population level, I choose example of meiosis genes evolution in a polyploid *Arabidopsis arenosa* (Brassicaceae) species complex. This only diploid-autotetraploid member of the plant leading model genus *Arabidopsis* provides an ideal system for addressing general questions on the triggers and consequences of genome duplication in plants. In contrast to other members of the genus, *A. arenosa* remained almost completely neglected by evolutionary biologists for a long time and only recently first studies emerged showing strong evidence of selective sweeps in genes connected with meiotic stability. They suggest that even generally highly conserved processes as the meiosis is are able to evolve quickly, when necessary. Understanding all that, the principal question still remains: does consequences of autopolyploidization in model *A. arenosa* species presents more challenge or benefits?



**KEY WORDS:**

*Arabidopsis arenosa*, autopolyploidy, genetic redundancy, meiosis in polyploids, polysomic inheritance, selection, selective sweeps

# ABSTRAKT

Autopolyploidie (znásobení celého genomu organismu) je náročná mutace. Přináší druhům, které ji prodělají, jak náročné výzvy, tak mnohé nové možnosti. Jako první se musí vypořádat s problémy, jako je ustálení nové linie v diploidní populaci rodičů, zajištění správného fungování buňky s dvojnásobným množstvím DNA a obnovení funkční mitózy a meiózy. Poté se však mohou projevit výhodné změny populační genetiky, jako je dvojnásobná efektivní velikost populace a polysomická dědičnost, které zvyšují heterozygotnost a genetickou variabilitu v nové polyploidní linii. Dále také snižují negativní působení genetického driftu a inbrední deprese. Z evolučního úhlu pohledu je patrné, že vlastnictví jednoho genomu navíc umožňuje selekci, aby působila na geny mnohem volněji. Alely si tak rychle rozdělí své dřívější funkce nebo získají funkce zcela nové. Abych lépe demonstrovala molekulární mechanismy působení selekce na populační úrovni, zvolila jsem jako modelový příklad evoluci genů pro meiózu u polyploidního druhu *Arabidopsis arenosa* (brukvovité, Brassicaceae). Je to jediný diploidně-autotetraploidní druh v rodě *Arabidopsis,* který je klíčovým rostlinným modelem. Jako takový *A. arenosa* umožňuje klást si obecné otázky ohledně příčin a důsledků celogenomové duplikace u rostlin. *A. arenosa* zůstával (na rozdíl od ostatních členů rodu) naprosto opomíjený evolučními biology. Až v poslední době se objevují studie, které ukazují významné známky selekce v genech, spojených se stabilitou jeho meiózy. Tyto studie naznačují, že i v evoluci silně konzervovaných znaků, jako je meióza, může dojít k rychlým změnám, když je potřeba. Když toto vše vezmeme do úvahy, vyvstává nám zcela zásadní otázka: představují důsledky autopolyploidizace u modelového druhu *A. arenosa* z evolučního pohledu spíše výzvu, nebo výhodu?



**KEY WORDS:**

*Arabidopsis arenosa*, autopolyploidie, nadbytek genetického materiálu, polyploidní meióza, polysomická dědičnost, selekce, selekční smetení

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# CONTENT

Introduction ..... 2
Polyploidy ..... 4
Definition allopolyploids vs. autopolyploids ..... 4
Polyploidy in the model species *A. arenosa* ..... 5
Autopolyploid formation: ..... 7
Selection in polyploids, focused on autopolyploidy ..... 9
Short-term changes after autopolyploidization ..... 9
Long-term changes after autopolyploidization ..... 11
    Polysomic inheritance and its implications for autopolyploid evolution ..... 11
    Genetic redundancy ..... 13
    Proliferation of transposable elements ..... 15
Autopolyploidy and answers to range shifts ..... 15
Null model of the polyploidy ratchet ..... 16
Introduction to the study system: the *Arabidopsis arenosa* complex ..... 17
Adaptation to autopolyploidy in plant genomes with a focus on meiosis ..... 20
Meiosis overview ..... 20
Adaptation to polyploid meiosis in *Arabidopsis arenosa* ..... 24
Conclusion ..... 30
Master thesis questions ..... 31
    1. Standing variation in the diploid lineages ..... 31
    2. Range-wide patterns of selection: does evolution repeat itself? ..... 32
    3. Meiosis pairing stability of the independent polyploid lineages ..... 33
Bibliography ..... 34

# INTRODUCTION

Polyploidy (whole genome duplication, WGD) plays a key role in plant evolution. An ancient gene duplication events have been identified in the extant ancestors of seed plants and angiosperms. WGDs probably led to the diversification of developmental regulatory networks, for example that of flower or seed development, thus facilitated establishment of new, evolutionary significant traits (Jiao et al, 2011). The importance of the polyploidy in the evolution of plants is additionally stressed by the finding of 23 ancient WGDs in land plants genomes (Garsmeur et al., 2014) and evidence of at least one polyploidization event in up to 95% monilophytes and 70% angiosperms (Soltis & Soltis, 1999).

Knowledge of plants' responses to WGD can facilitate the understanding of polyploid cancer cells, problems with human fertility or spontaneous abortions (reviewed in Wright et al, 2014). In plants it can contribute to crop improvement as polyploidy generates novel genetic variation, and, through doubling of DNA content, increases cell size and thus might theoretically modify various physiological functions (Renny-Byfield & Wendel, 2006, Bomblies & Medlung, 2014). Up to 75% of domesticated plants (so the majority of the food we eat) are polyploid (Hilu, 1993, Renny-Byfield & Wendel, 2014).

Autopolyploidy, whole genome duplication within a species, is generally characterized by polysomic inheritance, multivalent chromosome association in meiosis and no prior differentiation in genotype, as it involves closely related genomes.

In contrast to allopolyploids, which often possess evolutionary benefit in transgressive traits arisen through connection of two divergent genomes, the direct adaptive advantage of autopolyploidy is still controversial (Parisod et al, 2010).

It is accepted that established autopolyploids have evolutionary opportunities by the possession of two genomes, which might relax evolution and allow further diversification and speciation of redundant alleles. Diversification and subsequent sub-functionalization may release genes from pleiotropy and possibly improve chances of the newly arisen autopolyploid to colonize and survive in new or rapidly changing environments (Otto, 2007, Parisod et al, 2010, Hollister, 2015).

Polysomic inheritance might provide principal advantage from a population genetics point of view, leading to higher heterozygosity, lower level of inbreeding depression, lowered genetic drift and doubled effective population size, which might improve autopolyploids' adaptability and evolutionary success. Higher heterozygosity ensures masking of deleterious recessive

alleles and thus decreases the level of inbreeding depression in autopolyploids. Doubled effective population size maintains genetic variability in the species, preserves populations from the negative effects of genetic drift and increases the probability of the formation of beneficial mutations. This implies (and has been modelled) that, under partial or whole dominance and in small or middle-sized populations, autopolyploidy can increase the rate of adaptive evolution in comparison to diploid species (Otto, 2007, Parisod et al, 2010, Hollister, 2015).

In contrary, autopolyploidization could be regarded as severe mutation with remarkable negative effects. Immediately after polyploidization an autopolyploid faces numerous difficulties connected with its establishment. One of the most striking problems is how to divide four almost homologous chromosomes equally into two cells, using the meiotic apparatus that was fine-tuned for a diploid organism. Many examples of successfully established autopolyploids demonstrated that autopolyploids can overcome the challenges of polysomic chromosome segregation (e.g., Wright et al, 2014). Although various ways how to deal with autopolyploid meiosis remain largely unexplored, few pioneer studies in this field highlight the solution which evolved in the *Arabidopsis arenosa* complex. This species complex comprises both diploid and autotetraploid cytotypes and is closely related to the well-characterized model species *A. thaliana* and *A. lyrata*, therefore offering an excellent opportunity to study the molecular evolution of autopolyploidy. Using genome scanning approaches and comparisons with annotated reference genomes of its close relatives, a group of genes involved in meiotic processes was identified to be under selection linked to ploidy differentiation (Yant et al, 2013, Wright et al, 2014). Frequencies of their functionally different alleles vary strongly between ploidy levels and thus show evidence of selective sweeps and evolution driven by need of well-functioning meiosis. Moreover, selection on meiosis genes was found not only in tetraploids but also in diploids - in those exposed to different environments, highlighting the importance of well-functioning meiosis for both ploidies. All eight adaptive genes encode proteins that have functions in chiasma formation and chromosome juxtaposition during prophase of meiosis I (Hollister et al, 2012, Yant et al, 2013, Wright et al, 2014). As current knowledge of meiosis evolution is based on a limited sampling of both diploid and autotetraploid lineages from part of *A. arenosa*‘s distribution range, further studies are required, which are based on a comprehensive sampling of lineages and a range-wide sampling of *A. arenosa*, as well as suitable examples from other species in order to test if evolution is repetitive. This might contribute to a better understanding of the ecological and evolutionary context of meiosis adaptation to autopolyploidy.

I find the topic of polyploid evolution extremely intriguing. Without genome duplication, we would not have three-color vision, functional immune system or effective protein-protein interactions (reviewed in Otto, 2007).

In this review, I mainly focus on autotetraploids, i. e. polyploids possessing four sets of homologous chromosomes. This restriction is adequate as *A. arenosa* is an autotetraploid and, generally, literature and ecological and evolutionary models are mainly available for tetraploids in plants.

The main aims of my bachelor thesis is to review what is known about meiotic adaptation to autopolyploidization in the model plant *A. arenosa*, and to describe the ways in which selection acts on autopolyploids after their establishment. That ought to put the theoretical basis for my future research and contribute to a better understanding of a crucial evolutionary phenomenon.

# POLYPLOIDY

## DEFINITION ALLOPOLYPLOIDS VS. AUTOPOLYPLOIDS

Stebbins (1971): "*any attempt to maintain a division of natural polyploids into two discrete categories, autopolyploids and allopolyploids, is more likely to confuse than to clarify a very complex system of interrelationships*."

Two types of polyploids can be distinguished according to factors such as the way of polyploid formation, genome constitution, and chromosome behavior during meiosis: auto- and allopolyploids. In the literature two main approaches of polyploid classification are represented. Kihara & Ono, (1926) first classified polyploids on the basis of their origin: autopolyploids form within species and allopolyploids form via hybridization of two different species, which is usually connected to genome duplication. This approach has been taken by Ramsey and Schemske and others (Ramsey & Schemske 1998, 2002, Otto & Whitton, 2000, Wright et al, 2014). Personally I found this stance problematic because of the impossibility to define "species". Stebbins (1947) was the first who used a genetic/cytogenetic approach. He described that autopolyploids usually have polysomic inheritance, multivalent association of chromosomes during meiosis I shortly after their establishment and no prior differentiation in the chromosomal sets (Stebbins, 1947, Parisod et al, 2010, Wright et al, 2014). On the other hand, allopolyploids have multivalent association only rarely and they are analogic to diploids in their cytogenetic behavior as they contain two diploid genotypes in one genome (Stebbins,

1947, Parisod et al, 2010, Wright, 2014). However, even this definition is not absolute as we know many examples of polyploids being in some aspects intermediate. Thus Stebbins (1947) defined also so-called segmental allopolyploids – they produce multivalents during meiosis I and have polysomic genetic ratios but not as often as autopolyploids. In addition, some chromosomes in segmental allopolyploids are similarly differentiated as in allopolyploids while others appear homologues as in autopolyploids (Stebbins, 1947, Sybenga, 1996). I consider the definition of segmental allopolyploidy not very clear and Stebbins (1947) even questioned the existence of stable segmental polyploidy in nature. Thus I agree with Stebbins (1947), that, in my opinion, we cannot clearly define autopolyploids and allopolyploids, because they occur naturally in a large number of intermediaries, which continually evolve and can be combined together. Any attempts to define polyploidy should therefore be treated with caution and consideration. However, in order to communicate science, I need some systematic classification for my bachelor thesis so I adopted the genetic/cytogenetic approach to define polyploidy, for the reasons explained above.

To conclude, diploid genomes are characterized by the possession of two copies of each gene, AA (which have homologs BB in different diploid species). Merging diploid genomes, two types of tetraploids may form: autotetraploid - AAAA or allotetraploid - AABB. As we have a continuum between strict auto- and allopolyploid, an intermediate AAA´A´ can evolve when genomes of two diploid progenitors are only partly differentiated (Levin, 2002, Parisod et al, 2010).

## POLYPLOIDY IN THE MODEL SPECIES *A. ARENOSA*

My model species *A. arenosa* can be diploid or autotetraploid. Polyploid *A. arenosa* shows tetrasomic inheritance, but bivalent formation, so that chromosomes segregate steadily during meiosis I. However, the pairs among four homologues (A1, A2, A3 and A4) are formed randomly, thus the tetrasomic genetic ratio is produced (Fig. 1, Table 1) (Wright et al, 2014). As the merged genomes in tetraploid linages of *A. arenosa* are similar, it can still be considered as autopolyploid.

***Table 1***: *Possible chromosomal pairing during Prophase I shown on example of chromosomes A and B.*

| Diploid | A1A2 |
|---|---|
| | B1B2 |
| Autotetraploid *A. arenosa* | A1A2 A1A3 A1A4 A2A3 A3A4 |
| | B1B2 B1B3 B1B4 B2B3 B3B4 |
| A multivalent forming autotetraploid | A1A2 A1A3 A1A4 A2A3 A3A4<br>A1+A2A3A4 A2+A1A3A4 A3+A1A2A4 A4+A1A2A3<br>A1A2A3A4 |
| | B1B2 B1B3 B1B4 B2B3 B3B4<br>B1+B2B3B4 B2+B1B3B4 B3+B1B2B4 B4+B1B2B3<br>B1B2B3B4 |

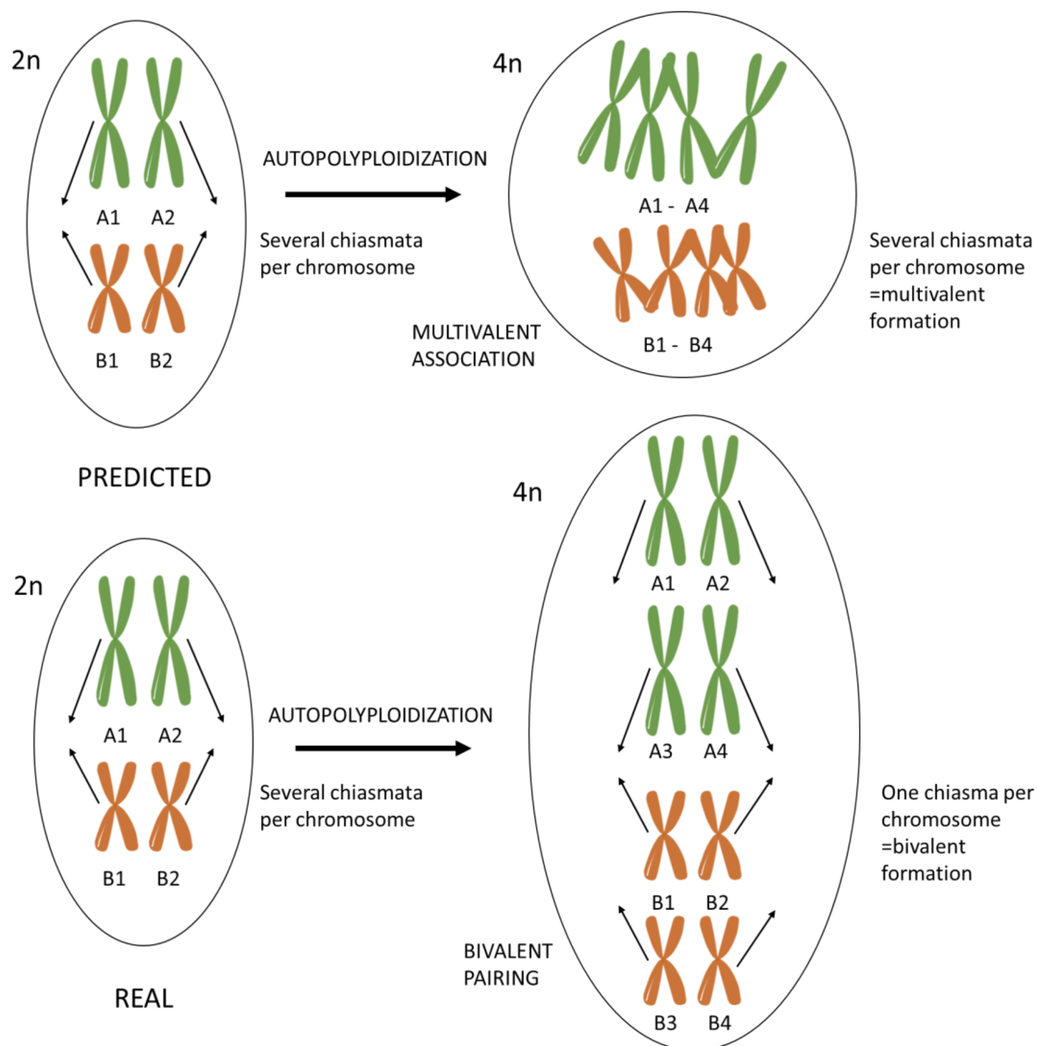


***Fig. 1***: *Chromosome segregation in A. arenosa autotetraploids. Arrows indicate direction of chromosome segregation in anaphase I. In tetraploids, bivalent pairing is random between the four chromosomes (see Table 1 for all possible combinations of chromosomes).*

## AUTOPOLYPLOID FORMATION:

There are three main ways how autopolyploids can develop. The less common is to form autopolyploids from somatic (thus primarily unreduced) cells which become polyploid (Fig. 2). However, this way of autopolyploid formation is rare. More often plants, which give rise to autopolyploids, produce unreduced gametes with two copies of each chromosome. The rate of unreduced gamete production is genetically determined and varies among species (Ramsey & Schemske, 1998, Parisod et al, 2010). It also depends on the environment; especially on stressful conditions such as herbivory, water and nutrient deficiency, solar radiation, heat or cold stress (Ramsey & Schemske, 1998, Husband, 2004, Parisod et al, 2010, Pécrix et al, 2011, Arrigo & Barker, 2012, De Storme et al, 2012, 2013). The unreduced gamete can combine with another unreduced gamete and directly produce autopolyploid progeny (Fig. 2). The process is sometimes called bilateral polyploidization (Parisod et al, 2010). Some studies propose that unreduced gamete formation is rather rare (Bomblies & Medlung, 2014). That is why unreduced gametes usually combine with normal haploid gametes and produce triploids. These can then combine with parental gametes or self-fertilize and produce both fully diploid and fully tetraploid genotypes and progeny (Fig. 2) (Ramsey & Schemske, 1998, Parisod et al, 2010). In his review, Levin, (2002) states that both ways of autopolyploid formation from unreduced gametes are equally plausible, because unreduced gamete production is much more common than previously thought (Maceira et al, 1993, Levin, 2002, Fawcett & Peer, 2010). One significant reason for that is existence of triploids. They often produce high number of aneuploid, diploid and triploid gametes and so they act as a kind of "polyploidy generators" in populations. This may be considered as more important reason for polyploid formation than stressful environmental conditions (Ramsey & Schemske, 1998, 2002).

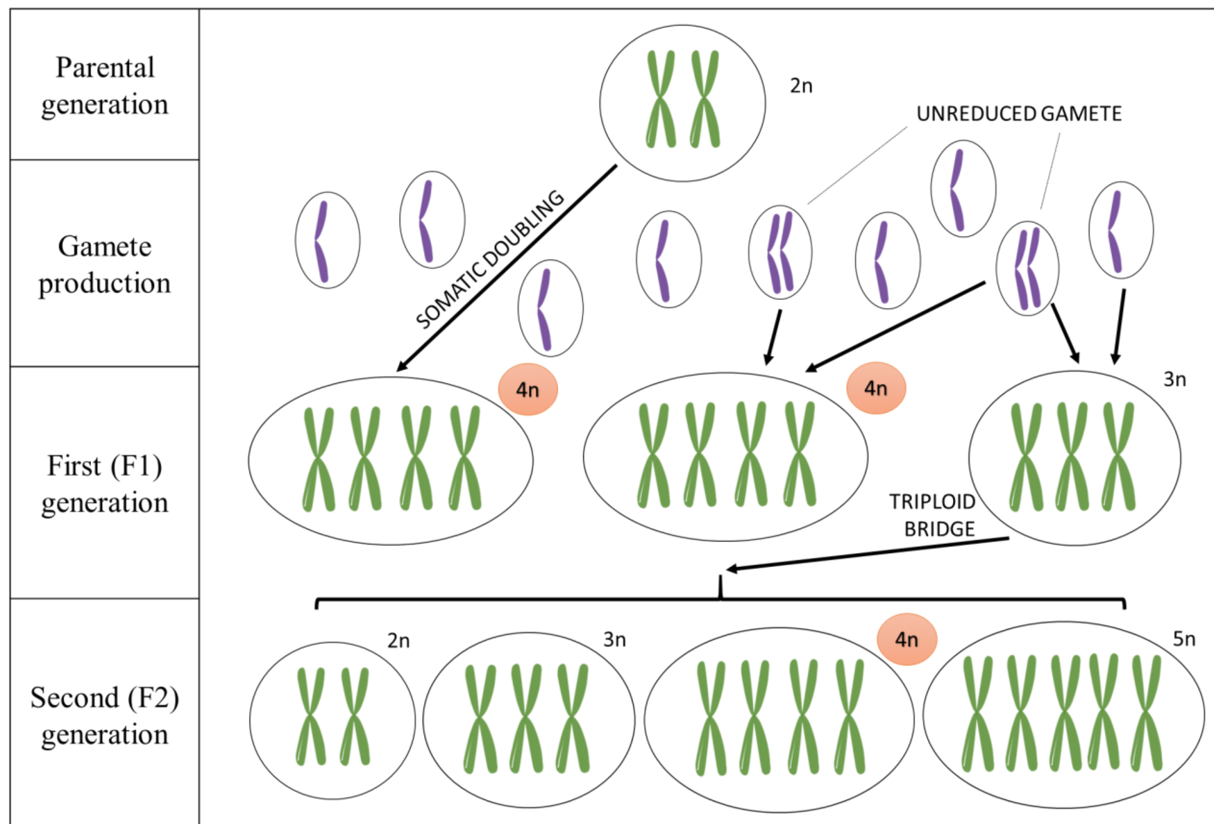


***Fig. 2:*** *Pathways of an autotetraploid formation. Three main ways how autopolyploids can develop are illustrated in Figure: somatic doubling, bilateral polyploidization and triploid bridge. In violet are gametes and in green somatic chromosomes. Gametes in the triploid bridge step are not shown.*

Allopolyploids can form in two main ways. The first way is via unreduced gametes from different species that fuse and give rise to an allopolyploid, but the frequency of unreduced gametes might generally be low (see discussion above about the origin of autopolyploids). Hybrid progeny can also form via fusion of normal haploid gametes from two different species (or divergent populations). Such hybrids are usually sterile because it is impossible to pair two different sets of chromosomes. This can be overcome with genome duplication which provides the needed pairing partners for chromosomes (Ramsey & Schemske 1998, Levin, 2002, Bomblies & Medlung, 2014).

# SELECTION IN POLYPLOIDS, FOCUSED ON AUTOPOLYPLOIDY

## SHORT-TERM CHANGES AFTER AUTOPOLYPLOIDIZATION

Immediately after polyploidization neo-autopolyploids face many challenges related to their survival and establishment.

First, autopolyploidization brings immediate challenges to the reproduction of the neo-autopolyploid individual. According to Hazzouri et al (2008), during autopolyploid establishment selection mainly acts on restoration of chromosome pairing from multivalents to diploid-like bivalents, reduction of the gene dose and amount of transposable elements by gene loss and breakdown of self-incompatibility (Hazzouri et al, 2008, Hollister, 2015). It has been observed that reduction of multivalent formation during meiosis is correlated with reduced chiasma frequency in neo-autopolyploids (for example in *Crepis capillaris*, *Hyoscyamus muticus*, *Lilium perenne* or *Lathyrus sativus*, as reviewed in Levin, 2002). This correlation may lead to reduced recombination in observed neo-autopolyploids (Levin, 2002, Hollister, 2015). It is important to mention (as discussed in the next chapter) that selection can either suppress or favor diploidization, depending on the circumstances, so the immediate-diploidization model is not the only plausible explanation.

Second, neo-autopolyploids arise within the diploid population, which is a rare or unique event, and thus frequency-dependent selection acts against the rare cytotype. This population-level effect on neo-autopolyploid establishment is called minority cytotype exclusion principle (Levin, 1975). It stems from reduced fitness of the rare neo-autopolyploids compared to its diploid ancestors due to unsuccessful pollination by prevailing pollen of the diploids (Levin, 1975, Husband, 2000, Hazzouri et al, 2008, Parisod et al, 2010). In the beginning, minority disadvantage can be overcome by assortative mating, selfing, apomixis or vegetative propagation. In addition, the triploid bridge discussed in the chapter about polyploid formation contributes to the coexistence of diploids and tetraploids in a population and helps with tetraploid establishment (Husband, 2004, Suda & Herben, 2013). Afterwards, a competitive advantage (discussed in the next chapter) and ecological divergence leading to niche differentiation and/or colonization of new environments, or, alternatively, stochastic processes favoring neo-autopolyploids are needed for the establishment of the new autopolyploid lineage (Otto & Whitton, 2000, Oswald & Nuismer, 2011, Bomblies & Medlung, 2014). The minority cytotype exclusion concept

forcing newly derived species into niche differentiation has been described for autopolyploids (Stebbins, 1975). However, I think that it can also be applied to allopolyploids to some extent as they may also face competition with their parental species. In contrast to autopolyploids, which I focus on, neo-allopolyploids have the advantage to possess two different genomes, which allows to generate extreme transgressive traits, which might appreciably facilitate ecological divergence (Rieseberg et al, 1999, Otto & Whitton, 2000). Possession of complementary genes, overdominance and epistasis are the primary cause of extreme transgressive phenotype in allopolyploid (Rieseberg et al, 1999).

Selection also favors changes leading to interploidal niche differentiation during autopolyploid establishment regarding, e.g., variation in temperature, light or moisture tolerance. Enzyme activity may be altered with the decrease of the surface–volume ratio of theoretically bigger polyploid cells and thus smaller relative area of membranes (Levin, 2002, Hollister, 2015). Another effect has the two times higher number of DNA to encode enzymes which can lead to an increase in gene-dosage-regulated enzyme production immediately after autopolyploidization (Hough et al, 2013). Selection favors these autopolyploidy-connected shifts as they may cause for example changes in mineral uptake efficiency, secondary metabolism or moisture tolerance and thus promote ecological differentiation of the newly arisen autopolyploid. A similar effect might have the predicted slower growth rate of polyploid plants (caused by the necessity to replicate a two times higher amount of DNA), which might change life history traits of the species from annuality to perenniality. Another change might be altered pollinator attraction (theoretically, polyploid species have bigger cells and thus may form bigger flowers, at least in the first few generations after polyploidization) (Levin, 2002, Hough et al, 2013). However these arguments are often rather theoretical considerations and empirically not well-proven. Interactions between genotype, DNA content and phenotype usually do not function straightforward, and especially empirical field studies are needed to assess these interactions. In particular we miss suitable model species systems for addressing effects of genome doubling *per se* from subsequent selection upon autopolyploids, thus the exact adaptive effect of autopolyploidy remains unclear.

We can only speculate to which extent processes, occurring immediately after polyploidization, are driven by selection (Soltis & Soltis, 1999, Yoo et al, 2014). They may partly be the consequence of polyploidization *per se*, i.e., molecular and functional change associated with ploidy increase itself, however, they still have to prove adaptable to be fixed. Another question is if the processes related to initial niche differentiation affect the way in which selection acts on autopolyploids later on.

## LONG-TERM CHANGES AFTER AUTOPOLYPLOIDIZATION

The otherwise progressive Stebbins (1971) stated that "*multiplication of chromosome sets either has little effect upon evolutionary progress at the gene level, or actually tends to retard it*". To challenge this sentence we need to review what is known about the evolution of autopolyploids and how they can respond to selection on a long-time scale.

After the successful establishment of a new, autopolyploid population, it has to face various environmental challenges. One of the most noteworthy questions regarding these challenges is whether autopolyploids respond more quickly (or more strongly) to selection than their diploid counterparts (or allopolyploids) (Ramsey & Schemske 2002, Parisod et al, 2010). Reviewing the literature to answer this question I concluded that three attributes of autopolyploidy should be taken into the account as some empirical studies proofed them to have a significant impact: polysomic inheritance, genetic redundancy and partially the proliferation of transposable elements. Although connected with each other, I will discuss the extent to which each of them contributes to the evolution of autopolyploids separately.

### POLYSOMIC INHERITANCE AND ITS IMPLICATIONS FOR AUTOPOLYPLOID EVOLUTION

As stated in previous chapters, autotetraploids possess four chromosome sets with random pairing in meiosis and thus have tetrasomic (or polysomic in autopolyploids) inheritance. In general, polysomic inheritance has many important implications for microevolutionary changes of a polyploid. First, it enables a higher level of heterozygosity in the populations (composed of partial - AAAa, Aaaa and complete - AAaa heterozygosity) (Ronfort, 1999). Furthermore, the loss of heterozygosity after inbreeding or selfing is much slower in autopolyploids than in a diploid population - which would be particularly important when the selfing rate is increased during autopolyploid establishment. Doubled genetic dosage also makes effective population size ($N_e$) of autotetraploids almost two times higher than is $N_e$ of their diploid progenitors (Ronfort, 1999, Arnold et al, 2012). That has two main implications for microevolution: (1) autotetraploids are less prone to genetic drift, and (2) the rate of mutation accumulation is theoretically two times higher. Doubled effective population size influences the mutation rate in a population (Otto, 2007): "*If mutations occur at rate $\mu$ per gene copy and each individual carries c gene copies ($c = 2$ in diploids; $c = 4$ in tetraploids), the equilibrium fitness of a population is reduced by $c\mu$. Thus, eventually, polyploids suffer more from recurrent deleterious mutations than diploids*". On the other hand, there are

theoretically twice as many beneficial mutations in a polyploid population, so the effect on fitness may not be one-sided. In general, autopolyploid populations may evolve more quickly than diploid ones when the beneficial mutations are (at least) partially dominant. Thus the rate of polyploid evolution will depend, among other aspects, on the extent to which mutant alleles are masked (Otto & Whitton, 2000, Otto, 2007).

Apart from the type of mutations the effective population size influences the rate of adaptation as well. As demonstrated in the yeast *Saccharomyces cerevisiae*, in asexual polyploid populations, beneficial mutations arisen in two individuals cannot be combined through recombination into one cell through sex (Anderson et al, 2003). Thus only beneficial mutations which arise in already stabilized cell lineages can survive and be favored. In large populations beneficial mutations arise quite often - no matter what the ploidy of the population is. However, as has been said, when they are partly or absolutely recessive, they are masked in asexual polyploids, thus they are favored more slowly or not at all if selection acts on them. On the contrary, in small populations beneficial mutations are rare, and the higher mutation rates in polyploids (as discussed above) can increase the adaptive rate in asexual populations and introduce needed variability. The increase in adaptive rate is prospective if beneficial mutations are not too much masked and at least partly dominant (Otto, 2007, Parisod et al, 2010). However, this strict masking of beneficial mutations is the case in completely asexual populations, which are very rare in plants. Thus the question remains, to which extent (if to any) these mutations play a role in plant autopolyploid speciation.

Last but not least, autopolyploids reach Hardy-Weinberg equilibrium gradually, in several generations, while diploids require only one generation to establish it (Levin, 2002). In contrary, autotetraploids need on average 3.8 generations of selfing to lose 50% of heterozygosity while diploids requires only a single one (Weiss-Schneeweiss et al, 2013). As we can see, polysomic inheritance and duplicated gene dose considerably change genetic variation and its distribution in an autopolyploid population (Ronfort, 1999, Weiss-Schneeweiss et al, 2013). More concretely, genetic variation measured as proportion of heterozygosity of a tetraploid outcrosser should be twice as high as that of a diploid with the same effective population size (Moody et al, 1993).

The effect of inbreeding depression (negative functioning of deleterious alleles in the population) in autopolyploids depends on the way in which negative mutations influence fitness. If they are dominant, autopolyploids face stronger inbreeding depression than their diploid counterparts (Husband, 2004, Parisod et al, 2010, Ramsey, 2011, Weiss-Schneeweiss

et al, 2013). However, this “overdominant” model is not well supported by empirical studies in nature (Ramsey & Schemske, 2002). On the other hand, the model of recessive mutations (when homozygous) negatively influencing fitness has been supported by many studies (reviewed in Husband et al, 2008, Parisod et al, 2010). In this case, inbreeding depression should be half as high in autopolyploids than in diploids because of a higher level of heterozygosity (see above) (Ronfort, 1999, Ramsey & Schemske, 2002, Otto, 2007, Weiss - Schneeweiss et al, 2013, Hollister, 2015). That implies that twice higher allelic dosage and richer allelic diversity can help autopolyploids to overcome inbreeding depression and thus provides immediate advantage in the competition with diploid species (Levin, 2002). Galloway & Etterson (2007), however, pointed out that not all empirical studies have to necessarily fit to this theoretical consideration. Factors such as the age of autopolyploid species, level of diploidization, difference between partial and complete heterozygotes (degree of dominance) and the gradual loss of allelic diversity have to be taken into account too. Gallway & Etterson´s empirical studies on autotetraploid *Campanulastrum americanum* even suggest that the rate of inbreeding depression could be almost the same in autopolyploids and diploids. Moreover, during their establishment, autopolyploids often produce offspring by self-fertilization for many generations. That can work against higher heterozygosity and deepen inbreeding depression, too. However, to fully understand these processes, more empirical studies are needed.

## GENETIC REDUNDANCY

“*Duplications enable genes to make evolutionary experiments which have previously been forbidden.* “ Kimura, (1983).

Polyploid genomes provides a high amount of redundant genetic material. This leads to relaxed selection on the redundant duplicated genes, and they can evolve more freely. Accumulation of negative mutations usually causes inactivation of the duplicated genes (Otto, 2007). However, some changes may be adaptable - they are retained and can be fixed. Adaptable changes of the duplicated genes comprise neo-functionalization (duplicates get new functions) or sub-functionalization (two duplicates divide their prior function and specialize on a sub-function). That is extremely useful in pleiotropic genes (genes which influence several phenotypic traits). By dividing multiple functions of one gene onto gene duplicates, polyploidization provides a possibility to escape pleiotropy and thus the speciation might act more effectively (Flagel & Wendel, 2009). This concept is described in allopolyploids, and Flagel & Wendel (2009) and Parisod et al (2010) speculate if we can also

apply it to autopolyploids, as we miss convincing evidence of duplicate diversification for them. However, when comparing synthetic and natural autopolyploids we observe differentially adapted genomes (Parisod et al, 2010). This can be the result of connecting two slightly divergent parental genomes during natural autopolyploid formation, or it is the consequence of adaptive changes after autopolyploidization, supporting relaxed speciation of duplicated genes (Flagel & Wendel, 2009). Increased initial diversity of neo-autopolyploids under natural conditions in comparison to synthesized ones has been observed in *Biscutella laevigata* (Parisod & Besnard, 2007) thus supporting differentiation of slightly variant duplicates (Parisod & Besnard, 2007, Parisod et al, 2010).

Regarding the evolution of duplicates, maintenance of dosage-sensitive gene balance has to be taken into account. If polyploidization ends in dosage imbalance in a gene regulatory network, it may decrease efficiency of such genes and thus reduce the fitness of the plant. The gene-dosage balance theory led to the finding that, regarding polyploidization, we have to treat genes (or better loci) as single units, which are subject to selection and evolution independently (Flagel & Wendel, 2009).

To conclude, as summarized by Parisod et al (2010): „*genetic redundancy could potentially facilitate adaptive divergence of duplicated genes, increasing the long-term genome flexibility of autopolyploids and favoring their retention. However, the efficiency of selection and the long-term adaptive potential of autopolyploids remain largely unexplored*". In her review Otto (2007) submits arguments that intensify Parisod´s careful statement, asking why homeologs are retained in polyploid genomes when mutations leading to gene inactivation and loss are so common. Her answer is that duplicated genes have to play an important role in plant evolution, which prevents their elimination. This role can be neo- or sub-functionalization and immediate specialization, gene-dosage balance or heterozygosity maintenance, as discussed above, or, for example, selection for increased gene expression levels (Otto, 2007). All these functions mediated with a twice as high gene dose and relaxed selection may possibly contribute to the evolutionary success of autopolyploid species, and I believe they are one of the main selective benefits in the evolution of autopolyploids compared to diploids. Or, at least, the duplicated genes after polyploidization are considered to be major facilitators of evolutionary change by providing the raw material for selection and further evolution (Otto, 2007).

### PROLIFERATION OF TRANSPOSABLE ELEMENTS

Another factor possibly contributing to enhanced autopolyploid speciation has been described: transposable elements. Polyploidization permits massive proliferation of transposable elements through breakdown of plant silencing mechanisms and masking deleterious recessive mutations (Adams et al, 2003, Adams & Wendel, 2005, Hazzouri et al, 2008). This is mainly the case in allopolyploids, but masking of recessive mutations occurs also in autopolyploids. There may be a higher rate of accumulation of transposable elements in autopolyploids compared to diploids, however the fixation of them is more probable in allopolyploids as autopolyploids miss distinct homeologous locus thus has tetrasomic inheritance (Hazzouri et al, 2008). Proliferating transposable elements enhance further diversification and duplication of genes, alteration of regulatory networks, genome reorganization and, according to Hazzouri et al (2008) and Flagel & Wendel (2009), significantly contribute to the generation of evolutionary novelty induced by polyploidization. As was written by Wessler & Carrington (2005): "*polyploidy doubles the number of cards in the deck, and, through transposon release, could initiate the process of shuffling as well*"

## AUTOPOLYPLOIDY AND ANSWERS TO RANGE SHIFTS

Although this text should deal with general mechanism rather than particular ecological questions, one of such a questions is so strongly associated with polyploid evolution that it deserves our attention: does autopolyploidy bring advantages in environments which underwent rapid change? The answer lies in two features that are consistently connected to autopolyploidy: genetic redundancy, which permits mutational robustness and speciation to behave more freely, and polysomic inheritance (Oswald & Nuismer, 2011). A study of the Brassicaceae species *Biscutella laevigata* (from a single maternal lineage) showed advantages of autopolyploidy connected with colonization of new habitats during environmental change, and how polyploidy helped to deal with the founder effect (Parisod & Besnard, 2007). In contrast to diploids, autotetraploid populations of *B. laevigata* expanded along an altitudinal gradient after recolonization of the deglaciated areas in the Alps, maintaining its genetic diversity due to the high heterozygosity level of autopolyploids, and thus were not subjected to the founder effect. In general, autopolyploids are less inclined to genetic drift due to higher effective population size and heterozygosity which are consequences of tetrasomic inheritance, as discussed above (Parishod & Besnard, 2007).

In conclusion, autopolyploidy proofs to be beneficial under changing conditions or in heterogeneous environment. It can overcome genetic depauperation following genetic drift and selfing in small populations. Higher probability of unreduced gamete formation in the stressful environment is another factor for the observation of more polyploids under these conditions. The adaptive potential of two redundant chromosomal sets may contribute to the evolution of new traits and, theoretically, longer persistence of a population in its locality. Compared to allopolyploids, autopolyploids lack the short period off drastic chromosomal changes and structural rearrangement after polyploidization. However, only few publications dealing with adaptable potential of autopolyploidy are available. Parisod et al (2009) hypothesize that, due to reasons described above, polysomic inheritance is beneficial and thus adaptive. That is the reason why massive chromosomal changes, which can lead to diploidization, are not strongly selected. Furthermore, genome doubling does not provide considerable genetic novelty, so autopolyploids lack new transgressive traits for selection to act on.

On the other hand, in the long-time scale, polysomic inheritance may be disadvantageous because it masks deleterious recessive mutations and opposes efficient selection in some aspects. As a result, it is retained in the short term, during e. g. climate-induced range shifts, but the level of polysomic inheritance is reduced along the evolutionary time scale, leading to diploidization. Adaptive genetic variability formed under genetic redundancy is however retained. Therefore diploidized autopolyploids (paleopolyploids, ancient polyploids) are common among plants, however they are hard to identify and we need to use large-scale genome sequencing approaches to clearly recognize them.

## NULL MODEL OF THE POLYPLOIDY RATCHET

In 2006, Meyers & Levin proposed that polyploids do not necessarily need to show either increase in adaptability or direct advantages to establish and persist in a diploid population. They explained, assuming that polyploidy is generally irreversible, polyploids simply exist because after the polyploidization, which can be an accidental process that cannot immediately return back to the diploid state (Meyers & Levin, 2006). This model is called polyploidy ratchet and is thought of as null model for polyploid evolution (Hollister, 2015). It has been supported with Oswald´s model of establishment and coexistence of different ploidy levels in a population. However the model has its weaknesses as it is based on the assumption

that polyploids establish rarely and omits the situation when polyploids establish in the whole geographical range and entirely extrude diploids (Oswald & Nuismer, 2011).

Although we discussed a variety of different opinions of autopolyploid adaptation and evolution and we still need to answer many questions regarding selection and evolutionary pathways in them. Based on our current knowledge, we can substitute Stebbins sceptic statement from the introduction of the chapter with another, rather emphatic one (Flagel & Wendel, 2009): *"duplication is truly the 'stuff of evolution*"

And from Otto & Whitton (2000): *"...polyploids are either not monsters or happen to be particularly hopeful monsters.* "

And for our model species, *A. arenosa* (Kolář, 2015): "*In the Arabidopsis arenosa group (...) (auto) polyploidisation is a major diversification force in the complex, generating an intricate mixture of diploid populations and their tetraploid derivatives*"

# INTRODUCTION TO THE STUDY SYSTEM: THE *ARABIDOPSIS ARENOSA* COMPLEX

The *A. arenosa* complex is considered to be an especially attractive model for studies of molecular ecology and evolution in autopolyploids as it comprises diploid (2*n*=2*x*=16) and autotetraploid (2*n*=4*x*=32) cytotype and is closely related to A. *thaliana* and *A. lyrata*, for which a rich literature in molecular biology, physiology and ecology exists. Furthermore, the evolutionary history of this species complex was recently reconstructed (Schmickl et al., 2012). *Arabidopsis arenosa* is a herbaceous plant from the family Cruciferae (Brassicaceae) with white or pink flowers, a leaf rosette and plant height ranging from 5 to 50 cm (Fig. 3a, b) (Májovský & Krejča, 1964, Al-Shebhaz & O'Kane, 2002, Slavík & Hejný, 2003). It is mainly outcrossing, annual, biennial or perennial (with biennial life cycle predominant) (Fig. 3a, b). Despite virtual absence of extant ploidy-mixed populations (Kolář et al, 2015), traces of relatively frequent interploidal gene flow have been detected (Jørgensen et al, 2011, Schmickl et al, 2012, Bomblies & Medlung, 2014) possibly in both directions (Arnold et al, 2015).

***Fig. 3a:*** *Flowering mountain ecotype of A. arenosa (recognized as Arabidopsis neglecta). Southern Carpathians, Fagaraš, Romania, photo F. Kolář, 21/7/2014.*
***Fig. 3b:*** *A. arenosa leaf rosette under snow, Western Carpathians, Nízké Tatry, photo D. Bohutínský, 1/1/2015.*

*Arabidopsis arenosa* is clearly an autotetraploid, due to its random pairing of homologous chromosomes in meiosis (Fig. 1, Table 1) (Hollister et al, 2012, Yant et al, 2013), no prior differentiation of homologs and close genetic proximity to their putative diploid ancestors (Schmickl et al, 2012, Bomblies & Medlung, 2014, Arnold et al, 2015). Interestingly, *A. arenosa* tetraploids lack, despite random pairing of chromosomes, multivalent chromosome association. Instead, they show "normal" bivalent pairing (Hollister et al, 2012), which probably evolved to overcome problems with unequal multivalent segregation and aneuploidy after the meiosis (Fig 1, Table 1). Therefore, meiosis in *A. arenosa* is cytologically diploidized, but genetically tetraploid. That means it shows patterns of polymorphism of diploid populations with twice the effective population size. That is why we can use statistical methods for diploids to search for selective sweeps in the autotetraploid genome (Hollister et al, 2012, Wright et al, 2015).

The limitation of the crossover (CO) number to one in autotetraploid *A. arenosa* is the proposed mechanism for stabilization of the diploid-like meiosis (Yant et al, 2013). If the number of CO is reduced to one, multivalents cannot be created and bivalent formation is ensured. Empirical studies on *A. arenosa* support this hypothesis. In diploids, the ratio of one and of two crossovers per bivalent is 1.6:1, while the same ratio in natural autotetraploids is 7.5:1 (Yant et al, 2013).

The *A. arenosa* complex inhabits a wide geographical and ecological range from sand dunes near the Baltic Sea to high-alpine habitats in the Alps and Carpathians (Schmickl et al, 2012, Kolář et al, 2015). Approximately nine taxa have been recognized within the complex so far, although they do not correspond with the recently reconstructed evolutionary history (Schmickl et al, 2012, Arnold et al, 2015) and the internal taxonomy of the complex is highly provisional. The genetic diversity center and probable origin of the widespread tetraploid lineage is situated in the Western Carpathians, where diploid and autotetraploid populations meet along an altitudinal gradient (Schmickl et al, 2012, Arnold et al, 2015). Similar to the Western Carpathians, cytotypes also overlap in the Slovenian Forealps and in Southern and Eastern Carpathians. Apart from these three zones of spatial overlap, cytotypes of *A. arenosa* show parapatric distribution with tetraploids inhabiting the central and northern part of Europe (approximately two thirds of the total distribution area) and diploids being found in the southeastern part of Europe and along the southern Baltic Sea coast (Kolář et al, 2015). The northern part of the *A. arenosa* areal, mostly inhabited by autotetraploids, has been formerly glaciated. That supports the hypothesis about autopolyploids colonizing and surviving in new or rapidly changing environments, shown, for example, in *Biscutella laevigata*, (Parisod & Besnard, 2007). Ploidy-mixed populations are extremely scarce, and the populations usually show cytotype uniformity even in the zones of cytotype overlap (Kolář et al, 2015).

To conclude, *A. arenosa* is a highly promising model for studies of molecular evolution in autopolyploids and gene adaptation to autopolyploidy. First of all, while majority of the textbook examples of polyploidy are in fact polyploid hybrids (allopolyploids), *A. arenosa* shows particularly unique example of clear autopolyploid. That makes *A. arenosa* beneficial for studying distinct effect of polyploidy, without effect of hybridization, on plant evolution (Bomblies & Medlung, 2014). It also allows fully benefit from a wide spectrum of molecular tools developed for *Arabidopsis* useful for comparative analyses of genetic, genomic and molecular-ecological processes accompanying genome doubling (Bomblies & Medlung, 2014). Genomes of its close relatives *A. thaliana* and *A. lyrata* have been assembled and annotated, thus we can use them as references for *A. arenosa*, which greatly facilitates work with genomic data. The ease of making colchicine induced, artificial neo-autotetraploids from the diploid plant facilitates to use those as a negative control (Yant et al, 2013).

# ADAPTATION TO AUTOPOLYPLOIDY IN PLANT GENOMES WITH A FOCUS ON MEIOSIS

Whole genome duplication represents, from the molecular point of view, a dramatic mutation. Apart from short-term solutions, such as changes in gene expression and epigenetic regulation, long-term polyploid genetic stabilization has been observed (Storchova et al, 2006, Hollister et al, 2012, Wright et al, 2014). Although there is evidence that selection and adaptive evolution after autopolyploidization occurs across kingdoms (Comai, 2005), very little is known about the principles and molecular mechanisms which underlie it. First studies dealing with those in plants used the *A. arenosa* diploid-autotetraploid species complex as a model (Hollister et al, 2012, Yant et al, 2013, Wright et al, 2014). By searching genome-wide for selection in *A. arenosa* autotetraploids, functional classes of genes which recently underwent selective sweeps have been described (Hollister et al, 2012): Selection acts on genes affecting epigenetic regulation, basal transcription regulation, homologous recombination, cohesion of sister chromatids, DNA repair, cell cycle and morphogenesis and cell growth (Hollister et al, 2012). Studies on autotetraploid yeast showed that the changes detected in *A. arenosa* are congruent with that of yeast and thus are shared across kingdoms (Storchova et al, 2006), which implies that these new functions might be generally adaptable in autotetraploids (Hollister et al, 2012).
I will from here on focus on adaptive changes connected to polyploid meiosis, as these are the most striking and best studied changes up to now (Hollister et al, 2012, Yant et al, 2013, Bomblies & Medlung, 2014, Wright et al, 2014), and this will be the topic of my diploma thesis. First of all, I generally summarize the process of meiosis and then I review what has been found about adaptation to polyploid meiosis in the model species complex *A. arenosa* so far.

## MEIOSIS OVERVIEW

Meiosis is a key biological process which is necessarily connected to sexual reproduction (Dawe et al, 1994, Blat et al, 2002, Hamant & Cande, 2006, Cole et al, 2010, Osman et al, 2011, Zhou & Pawlowski, 2014). This specialized type of cell division is characterized by a single round of DNA replication that is followed by two consecutive nuclear divisions (meiosis I and meiosis II), which reduce the chromosome number to one half. This means that meiosis produces four daughter cells with half of the number of chromosomes of the original

parental cell (Dawe et al, 1994, Blat et al, 2002, Hamant & Cande, 2006, Cole et al, 2010, Osman et al, 2011, Zhou & Pawlowski, 2014).

Before the cell can enter division (mitosis or meiosis) it has to grow and prepare in the so-called interphase, which is divided into the $G_1$, S and G2 phase. In the G1 phase the cell increases its size, obtains nutrients and divides organelles. The S phase is characterized by DNA replication and the $G_2$ phase by an active preparation for cell division. The interphase is the "living" part of the cell cycle and cells spend the majority of time in it (Snustad & Simmons, 2008).

As stated above, meiotic cell division consists of meiosis I and meiosis II. During meiosis I homologous chromosomes segregate and two haploid daughter cells are produced (Hamant & Cande, 2006, Osman et al, 2011). Because ploidy is reduced from diploid to haploid, meiosis I is called reductional division. Meiosis II is a division similar to mitosis, in which the sister chromatids are divided, and four haploid daughter cells are created (Hamant & Cande, 2006, Osman et al, 2011).

In seed plants (Spermatophyta), male meiosis occurs in the anther and female meiosis in the ovary (Osman et al, 2011, Zhou et al, 2014). Parental cells undergo the G1-phase of the cell cycle and continue to the S-phase. During S-phase, the DNA is replicated (Osman et al, 2011). Each of the chromosomes duplicates, so that it becomes a complex of two identical sister chromatids. Attachment of the sister chromatids is established by the cohesin protein complex (Fig. 5) (Hamant & Cande, 2006, Zhou & Pawlowski, 2014). During meiotic cell cycle, the G2-phase usually cannot be visually distinguished from the beginning of the M-phase, which is considered to be the beginning of meiosis (Hamant & Cande, 2006).

The first meiotic division, meiosis I, separates the pairs of homologous chromosomes, each with two sister chromatids, into two cells. One haploid set of chromosomes ends up in each of the two new daughter cells. Meiosis I consists of four stages – prophase I (which is further divided into leptotene, zygotene, pachytene, diplotene and diakinesis), metaphase I, anaphase I and telophase I (Blat et al, 2002, Hamant & Cande, 2006, Cifuentes et al, 2010, Cole et al, 2010, Osman et al, 2011).

Initiation of meiosis can be cytologically recognized at prophase I. Prophase I is the longest phase of meiosis, during which homologous recombination occurs. The first stage of prophase I is the leptotene, during which chromosomes condense and coil into visible arrays within the nucleus (Hamant & Cande, 2006, Osman et al, 2011). During the second, zygotene, stage chromosomes line up to homologous chromosome pairs. One of the biggest unresolved questions regarding the molecular mechanism of meiosis is to understand the mechanism

which allows homologous chromosomes to find each other and pair. Theories about recognition of the specific chromosome morphology via binding proteins or, more probably, by recognition of a signal sequence in the telomeric or sub-telomeric region have been proposed (Hammant & Cande, 2006, Calderón et al, 2014). The process called „bouquet formation“ may support the active clustering according to a specific marker. During bouquet formation, telomeres attach to the nuclear envelope and cluster to each other with the help of microtubules. Hamant & Cande, (2006) hypothesized that although pairing and bouquet formation are mutually independent processes, the clustering of telomeres is one of several possible mechanisms that may facilitate the initial homology recognition (Hamant & Cande, 2006, Osman et al, 2011).

After the recognition of homologous chromosomes, the synapsis follows. It consist of pairing and coupling of chromosomes via the protein structure synaptonemal complex (Fig. 5). Pairing works in a zipper-like fashion and is highly specific and exact, because both pairing chromosomes should be equal in length and position of the centromere. Zipping of the chromosomes is made by a transverse filament protein that polymerizes between them. The paired chromosomes are called bivalent, and in diploid species they create tetrads (Hamant & Cande, 2006, Osman et al, 2011).

The pachytene stage is the part of meiosis in which CO occurs. Non-sister chromatids of homologous chromosomes exchange segments of homologous DNA. CO formation is initiated by the formation of double-strand (ds) breaks. The key role in the break formation plays the *SPO11* protein (Fig. 5) (Blat et al, 2002, Cole et al, 2010, Zhou & Pawlowski, 2014). One strand of ssDNA (single-strand DNA) on one side of the break invades the homologous double-strand DNA of one of the two non-sister chromatids and forms an intermediate, so-called D-loop. This enables the capture of the 3′-end on the other side of the break. The ligation of the broken DNA strands is followed by the formation of the double junction (so- called Holliday junction, Fig 5). This recombination intermediate is resolved to form CO (Cole et al, 2010). However, studies in *A. thaliana* and other species suggest that the Holliday junction can end as non-crossover product too (Blat et al, 2002, Osman et al, 2011). At the sites where CO exchange happens, chiasmata (sites of recombination) form (Hamant & Cande, 2006, Osman et al, 2011).

In diplotene, the synaptonemal complex falls apart, homologous chromosomes partly separate from each other and homologous juxtaposition ends. However, the homologues are still held together as bivalents until metaphase I by chiasmata, the regions where crossing-over occurred (Hamant & Cande, 2006, Osman et al, 2011). Than diakinesis takes place,

the chromosomes condense further, thicken, and detach from the nuclear envelope. Sites of crossing over entangle together, making chiasmata clearly visible. Homologs later separate during anaphase I as the cohesin proteins are removed from the chromosomes and chiasmata disassemble. The rest of prophasis I mostly resembles prometaphase of mitosis. The nucleoli disappear, the nuclear envelope disintegrates into small vesicles and the cytoskeletal meiotic spindle begins to form (Hamant & Cande, 2006, Osman et al, 2011).

During metaphase I, kinetochore microtubules from both centrioles attach to the chromosomal kinetochores and the homologous chromosomes align along an equatorial plane. The plane divides the spindle into half, due to continuous counterbalancing forces of spindle microtubules pulling two kinetochores from homologous chromosomes. Independent assortment of chromosomes is achieved due to the random orientation of each bivalent along the metaphase plate. As spoken above, the cohesin protein complex holds sister chromatids together from the meiotic S-phase (replication) until anaphase. The cell cycle control point between metaphase I and anaphase I does not allow to progress with anaphase until all the chromosomes are properly connected to spindle microtubules and bi-oriented. This requires at least one CO site per chromosome pair to hold homologous chromosomes in addition to cohesion mediated holding between sisters chromatids (Petronczki et al, 2003, Cifuentes et al, 2010). During anaphase I the protein separase catalyzes the dissociation of cohesin from chromosomes and the sudden segregation of sister chromatids to the opposite poles of the cell. Kinetochore microtubules degrade at the ends, pulling homologous chromosomes to the opposite sides of the cell, while non-kinetochore microtubules lengthen and push homologous chromosomes farther apart. Unlike in mitosis, the cohesin surrounding the centromere remains protected. This is necessary because sister chromatids have to stay together while homologs are segregated (Petronczki et al, 2003, Snustad & Simmons, 2008).

The first meiotic division ends in telophase I, when the chromosomes (consisting of two sister chromatids) end at the cell poles. Each future daughter cell has the haploid number of chromosomes, but in contrast to mitosis each chromosome consists of two chromatids. An envelope surrounds both the new haploid nucleus and the chromosomes uncoil back into chromatin (Snustad & Simmons, 2008).

In case of mitosis, S-phase and replication of DNA follow cell division. In meiosis DNA replication is suppressed after the first division to maintain the haploid nucleus (Petronczki et al, 2003).

Meiosis II is the second part of the meiotic division, functionally similar to mitosis. The difference is in the genetic results - two diploid cells after mitosis versus four recombined

haploid cells (gametes) after meiosis in case of a diploid organism (Petronczki et al, 2003, Snustad & Simmons, 2008, Harrison, 2010). In case of tetraploids, mitosis results in two tetraploid daughter cells while meiosis in four diploid daughter cells. Meiosis II consists of four stages: prophase II, metaphase II, anaphase II, and telophase II, all of them are analogous to the mitosis stages (Snustad, 2008). In prophase II the nuclear envelope dissolves, chromosomes condensate and the meiotic spindle is prepared. Metaphase II is characterized by attaching chromatid kinetochores to spindle microtubules at each pole. Interestingly, a new equatorial plate is created and rotated by 90 degrees in comparison to the meiosis I equatorial plate (Snustad, 2008, Harrison et al, 2010). During anaphase II, the sister chromatids segregate into new daughter cells. Meiosis II ends with telophase II, which is very similar to telophase I and is characterized by decondensation of the chromosomes, disassembly of the spindle and formation of the nuclear envelope from vesicles.

## ADAPTATION TO POLYPLOID MEIOSIS IN *ARABIDOPSIS ARENOSA*

Genes encoding proteins which affect core meiosis processes are mostly well-conserved among eukaryotes, because correct processes of meiotic division, DNA recombination and chromosome segregation are essential for survival of almost all eukaryotic species. We can expect that such conserved, basic features would not show significant variation among species and even less variation among (and within) populations (Harrison et al, 2010, Wright et al, 2014). Surprisingly enough, recent studies showed that this is not always the case, because some meiosis genes, especially those which code proteins mediating prophase I processes, are sometimes divergent - not only on the species level but among populations, and in some cases even within them (Hollister et al, 2012, Yant et al, 2013, Wright et al, 2014). Cytotype-associated genetic adaptation to genome-doubling is well exemplified in the *A. arenosa* species complex. It is important to stress that *A. arenosa* is unique well documented case of meiotic selection after the autopolyploidization across the whole plant kingdom (Hollister, 2015).

Hollister et al (2012) tested twelve tetraploid individuals from four different habitats for habitat or population structure-associated differentiation. Pairwise $F_{ST}$ comparisons have been used across the genome, and they found low differentiation among populations. Thus low habitat-driven differentiation can be expected. To identify genes under selection (or with evidence of past selective sweeps) genes with (1) low polymorphism and (2) site frequency

spectrum skewed toward high frequency derived haplotypes have been found (Hollister et al, 2012). 192 genes under selection have been found in the genome of these autotetraploid *A. arenosa* samples. Based on the comparison to the annotated *A. lyrata* and *A. thaliana* genomes, functional categories have been assigned to these genes, and a. set of 70 genes with good evidence of direct involvement in meiosis have been identified amongst them (Hollister et al, 2012).

In a subsequent study, ploidy-driven speciation in meiosis genes has been investigated among 2 diploid and 4 autotetraploid populations (Yant et al, 2013). In a pre-defined set of the 70 meiosis genes (Hollister et al, 2012), eight unlinked loci were found which met "*0.5% outliers in the distributions of three metrics:* $F_{ST}$, *the two-dimensional site frequency spectrum (2dSFS) and the 0.5% most negative values of linear regression residuals from the relationship between diversity and differentiation*" (Yant et al, 2013). Thus, selection appears to be linked with ploidy level differentiation for these eight meiosis genes (Yant, et al, 2013).

Recently, Wright et al (2014) tested the link between selection for meiosis genes (pre-defined in Yant et al, 2013) and ploidy level on broader sampling. Moreover, they tested if selection on these meiosis genes is different in diploids.

The *A. arenosa* genome was screened (using PoolSeq) on the sample of 6 autotetraploid, 6 Pannonian diploid and 6 Carpathian diploid populations. Following stringent criteria of three independent tests "$F_{ST}$, *G, a statistic that quantifies raw allelic differentiation accounting for variation in read coverage and DD, which measures the ratio between the difference in allele frequency between populations and diversity within a population to account for the positive relationship between diversity and differentiation*" (Wright et al, 2014) the eight meiosis genes detected in Yant et al, (2013) were identified again (with one exception of the SMC1 gene, which did not show significant differentiation) (Wright et al, 2014). The genes showed significant differentiation among autotetraploids, Pannonian and Carpathian diploids, indicating that meiosis genes evolved not only after autopolyploidization, but also in diploid populations (Table 2) (Wright et al, 2014).

The eight differentiated meiosis genes encode the proteins *ASY1, ASY3, MEI1, PDS5, ZYP1a, ZYP1b, SMC1* and *SMC3* (Yant et al, 2013, Wright et al, 2014). SMC3 is included only in the results of Yant et al (2013); Wright et al (2014) identified it as a gene with a weak differentiation pattern and detected the gene PRD3 instead. To clearly understand the function of these proteins and their position during cell cycle and meiosis see Fig. 5 and Table 2

(Hollister et al, 2012, Yant et al, 2013, Bomblies & Medlung, 2014, Wright et al, 2014). Patterns of differentiation are found in Table 3.

The genes which show evidence of selection in diploids vs. tetraploids (ASY1, ASY3, PDS5, ZYP and SYN1) (Table 3) all encode proteins which coordinate important events in early meiotic prophase I, such as sister chromatid cohesion, axis formation and synapsis (Table 2, Fig. 5) (Higgins et al. 2005; Hamant & Cande, 2006, Osman et al, 2011; Ferdous et al, 2012). *ASY1* and *ASY3*, better described in yeast as *Hop1* and *Red1*, help to form the core structural protein of the chromosome axes, which acts as a physical component of crossing over regulation (Blat et al, 2002, Hamant & Cande, 2006, Osman et al, 2011, Yant et al, 2013). *SYN1* is a homolog of the important human protein *Rec8*, which regulates homologous recombination - crossing over and interaction with cohesins and chromosome axes (Table 2, Fig. 5) (Lam et al. 2005, Osman et al, 2011). *ZYP1a* and *b* form the central filamental structure of the synaptonemal complex (Table 2, Fig. 5) (Higgins et al. 2005, Osman et al, 2011, Wright et al, 2014). The so far described proteins are all involved in crossing over regulation. That means that in tetraploid *A. arenosa* genes which encode them might undergo selection which results in a reduced crossing over number, because lower CO frequency contributes to meiotic stability in polyploids (it prevents the formation of multivalent association among the polyploid homologs) (Cifuentes et al, 2010; Bomblies & Medlung, 2014). Another differentiated gene, MEI1, encodes a protein that plays a role in post-crossing over meiotic DNA repair (Table 2, Fig. 5) (Grelon et al, 2003).

*SMC1* is involved in sister chromatid cohesion and interacts with the *SYN1/REC8* in yeasts (Lam et al, 2005). SMC1 shows evidence of selection between Pannonian and Carpathian diploids, while the SYN1 and ASY3 show evidence of selection only between ploidy levels (Wright et al, 2014). This parallel is very interesting, because it suggests that axis formation and its function in crossing over control and coordination is under selection two times independently, once between the different ploidy lineages and a second time in phylogenetically distinct diploid lineages (Wright et al, 2014).

***Table 2:*** *Function of the proteins encoded by the meiosis-related genes that are under strong selection in A. arenosa (Mathilde et al, 2003, Lam et al, 2005, Sanchez Morgan et al, 2007, Ferdous et al, 2012, Kuntal et al, 2014, De et al, 2014, Hollister, 2014, Zamariola et al, 2014).*

| Name | Function in complex | Individual function |
|---|---|---|
| SYN1 | Structural maintenance of chromosome (*SMC*) proteins, along with the *SYN1* form the cohesin complex that is responsible for sister chromatid cohesion, synaptonemal complex formation and the proper segregation of chromosomes. *SMC1*, *SMC3* and *SYN1* are localized in the nuclear matrix during G1 phase and establish cohesion of sister chromatids after the DNA replication in S-phase. | Plant homolog of yeast *REC8*, non-*SMC* subunits of cohesion complex, required for the linking of *SMC1* and *SMC3* and *SMC3* connection to meiotic chromosomes. |
| SMC3 | | Proteins consisting of five structural domains (N-terminal NTP-binding motif, a C-terminal box, two coiled–coil domains and a hinge domain). *SMC1* and *SMC3* form heterodimer. Studies suggest that *SMC3* protein has some other functions beyond cohesin formation. |
| SMC1 | | |
| ASY1 | Interacting proteins *ASY1* and *ASY3* favor inter-homolog recombination. | Consist of two domains *HORMA* (homolog of yeast *Hop1*) and Swirm. It establishes densely along the chromosomes in late G2 phase. *HORMA* domain associate with axial elements and contribute to double-strand break via coordinating key recombination pathway protein *DMC1*. |
| ASY3 | | Homolog of yeast *Red1*. Affects axial organization of *ASY1*, interacts with *HORMA* domain via coiled-coil domain, contribute to synaptonemal complex formation. |
| MEI1 | | Protein containing five C-terminus domains. Performs DNA repair (independent of *SPO11*-induced recombination repair) during meiosis. |
| ZYP1a, ZYP1b | Two paralogues of the transverse filament protein which contribute to axis formation and maintain a continuous synaptonemal complex and correct crossing over frequency. | |
| PDS5 | Precocious dissociation of sisters protein 5 (*PDS5*) binds on N-terminus of *Wapl* protein and they together forms "antiestablishment" and the "antimaintenance" complex, which cleave *SYN1* and *SMC3* bond in cohesin complex during Prophase 1. Its function have been largely studied in yeast and human but need further investigation in plants. | |
| PRD3 | | Has weak pattern of differentiation in *A. arenosa*. Direct accessory protein of *SPO11* which create double stranded breaks that initiate meiotic recombination. |

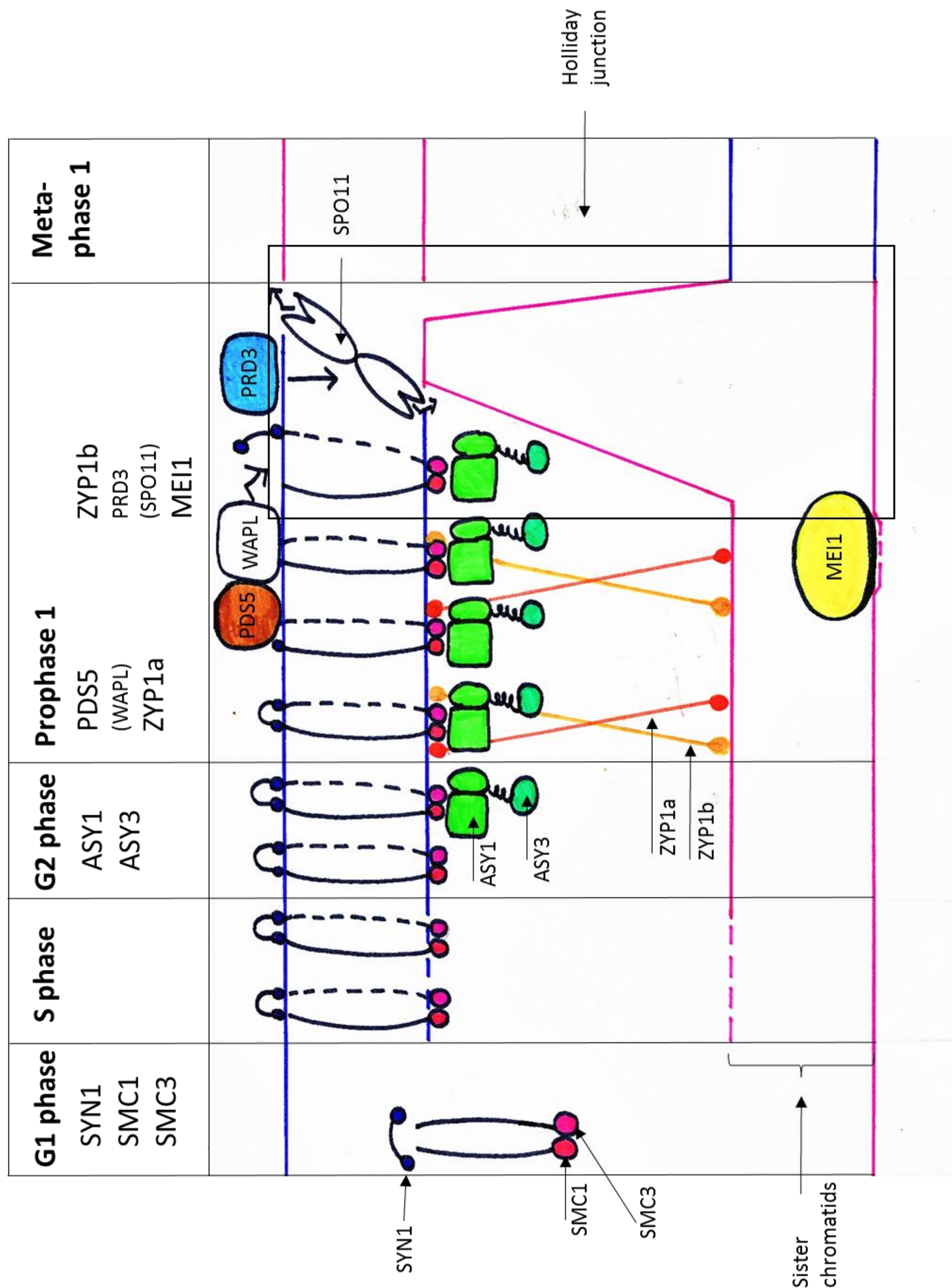


*Fig. 5: Function of the proteins encoded by the meiosis-related genes that are under strong selection in A. arenosa. Proteins with strong evidence of evolution are colored. SPO11 and WAPL are integrated for better understanding. PRD3 shows weak evidence of evolution (Mathilde et al, 2003, Lam et al, 2005, Sanchez-Morgan et al, 2007, Ferdous et al, 2012, Kuntal et al, 2014, Zamariola et al, 2014, Hollister, 2014).*

***Table 3:*** *Patterns of differentiation in meiosis-related genes that are under strong selection in A. arenosa with outlier values for* $F_{ST}$*, G, and DD statistic (Wright et al, 2014). Gene - indicates common name, ATG ID - the A. thaliana gene ID number, Contrast – contrast of population groups; H = Carpathian diploid, L = Pannonian diploid, Tet = tetraploid. Type – type of the differentiation; ALL – among all three groups, 2x – between diploids, 4x – between tetraploids.*

| Contrast | Gene | ATG ID | $F_{ST}$ | Gstat | DD | Type |
|---|---|---|---|---|---|---|
| H x L | ASY3 | AT2G46980 | 0.46 | 47.7 | 7.1 | ALL |
| H x Tet | | | 0.59 | 64.7 | 13.1 | |
| L x Tet | | | 0.66 | 65.4 | 9.0 | |
| H x L | SYN1 | AT5G05490 | 0.66 | 91.9 | 164.4 | ALL |
| H x Tet | | | 0.35 | 36.7 | 3.8 | |
| L x Tet | | | 0.85 | 103.6 | 8.5 | |
| H x L | SMC1 | AT3G54670 | 0.53 | 63.1 | 2.8 | 2x |
| H x Tet | | | 0.34 | 53.9 | 9.4 | |
| L x Tet | | | 0.46 | 51.1 | 7.4 | |
| H x L | MEI1 | AT1G77320 | 0.62 | 53.8 | 20.9 | 4x |
| H x Tet | | | 0.11 | 10.3 | 1.0 | |
| L x Tet | | | 0.54 | 42.2 | 13.7 | |
| H x L | ASY1 | AT1G67370 | 0.36 | 46.9 | 6.7 | 4x |
| H x Tet | | | 0.48 | 45.2 | 8.6 | |
| L x Tet | | | 0.53 | 59.8 | 11.0 | |
| H x L | PDS5 | AT1G77600 | 0.27 | 39.0 | 6.9 | 4x |
| H x Tet | | | 0.63 | 68.5 | 14.8 | |
| L x Tet | | | 0.56 | 57.4 | 15.9 | |
| H x L | ZYP1a | AT1G22260 | 0.17 | 14.7 | 1.5 | 4x |
| H x Tet | | | 0.31 | 40.2 | 1.8 | |
| L x Tet | | | 0.40 | 40.9 | 2.2 | |
| H x L | ZYP1b | AT1G22275 | 0.19 | 16.8 | 1.6 | 4x |
| H x Tet | | | 0.52 | 52.5 | 12.2 | |
| L x Tet | | | 0.51 | 49.4 | 7.9 | |

In addition to the results from Table 3 it is important to mention that regarding the ZYP1 gene, which has two functionally similar tandem duplicates ZYP1a and ZYP1b (Higgins et al, 2005, Ferdous et al, 2012), selection primarily acts on the ZYP1b paralog, suggesting that only this paralog may be the target of ploidy-associated selection (Wright et al, 2014). Five other genes (AT1G27900, MSH2, PRD3, SMC3, SMC6a) were described to have weak patterns of differentiation with ploidy level, and results for these five proteins were not consistent among different tests of differentiation or did not have a distinct pattern of selection (Wright et al, 2014). The rest of the predefined meiotic gene set (57 genes) did not have any evidence of differentiation and selection (Wright et al, 2014).

All the findings described above suggest that the set of eight meiosis-associated genes with strong differentiation in A. arenosa represent a multigenic, naturally evolved solution to

challenges that whole genome duplication, and thus polyploidization, present (Yant et al, 2013, Bomblies & Medlung, 2014, Wright et al, 2014).

The studies of Yant et al (2013) and Wright et al (2014) were focused on a subset of two diploid lineages from total (so far identified) six lineages (Kolář et al, unpublished) and one autotetraploid lineage from total three lineages (Arnold et al, 2015). Results from microsatellite analysis and preliminary results from restriction site associated DNA sequencing (RADSeq) data suggest, that an independent origin of autotetraploid lineages within diploid ones occurred three times in the *A. arenosa* complex (Kolář et al, unpublished). Age estimates of the origin of these lineages were not conducted yet, but we have strong evidence that the age of each autotetraploid lineage is different, and, therefore, we hypothesize that the degree of proper (diploid-like) meiosis establishment is different, too. That highlights the need for a follow-up comparative study of selection in recurrently originated autotetraploids.

The studies of Yant et al (2013) and Wright et al (2014) also omitted tetraploid populations from extreme conditions such as the high-alpine environment. Therefore patterns of differentiation and selection between different lineages and amongst and between ploidy levels and the role of environmental conditions herein remain partly unexplored, as they require integration of all evolutionary lineages and ecological conditions as well as a deep understanding of the evolutionary history of the model system. Especially the standing variation (genetic variation for alleles encoding traits which affect fitness in natural populations (Orr & Unckless, 2008) of meiosis genes in diploids needs to be evaluated. So far unexplored diploid lineages could bear preadaptations for polyploid meiosis or could shed light on speciation in diploids.

# CONCLUSION

In order to study selection in autopolyploids compared to their diploid progenitors, several factors need to be taken into account. Regarding the response on selection on recessive alleles, it is two times stronger in diploids, because they have a higher probability to form recessive homozygotes which can be eliminated if they bear deleterious mutations or can be favored if they bear beneficial mutations (Otto & Whitton, 2000, Weiss-Schneeweiss et al, 2013). Autopolyploids may form new gene combinations more slowly because of increased self-fertilization in some cases (Stebbins, 1950). However that does not necessarily mean that autopolyploids adapt more slowly than diploids. The level of self-fertilization can be actually

lower in polyploids than in diploids while inbreeding depression is suggested to be higher in autopolyploids than in diploids (reviewed in Mable, 2004). In small or middle-sized populations the rate of adaptive evolution depends more on the number of new mutations than the efficiency of selection, which is an advantage for tetraploids, because they undergo twice as high number of mutations than diploids because of their doubled effective population size (Husband & Sabara, 2004, Otto, 2007). Due to the higher effective population size, autopolyploids might also escape genetic drift and thus selection can be more effective in their relatively smaller populations. The rate of adaptive selection may even be faster for autopolyploids when beneficial alleles are dominant. Moreover, a significant part of the diploid genetic diversity is present in the autopolyploid gene pool and may be further increased by adding other diploid maternal lineages in case of a multiple autopolyploid origin (Husband, 2004) and/or across-ploidy admixture (Arnold et al, 2015). This might also contribute to the evolutionary success of autopolyploids. Finally, autopolyploidization can increase the level of adaptive evolution also via a higher chance to evolve new functions from duplicated genes (Otto & Whitton, 2000), and the adaptive genetic variability formed under the genetic redundancy is retained even after diploidization occurred in ancient polyploids (Otto & Whitton, 2000).

## MASTER THESIS QUESTIONS

To understand the general patterns of selection on autopolyploid genomes I decided to choose the set of meiotic genes being under strong selection for proper polyploid meiosis in *A. arenosa* as a model system (Yant et al, 2013, Wright et al, 2014). In the follow-up practical study I would like to deal with three main issues: 1. the assessment of the standing variation in diploid *A. arenosa* populations, 2. the comparison between differentiation and selection in recurrently originated autotetraploids and their diploid putative progenitors and 3. correspondence between meiosis pairing stability and level of establishment of the putative independent polyploid lineages with different level of population establishment.

### 1. STANDING VARIATION IN THE DIPLOID LINEAGES

Studies of meiosis adaptation to polyploidy in *A. arenosa* were so far based on the comparison between the Western-Central European autotetraploid lineage (Arnold et al, 2015) and Carpathian and Pannonian diploid lineages (Wright et al, 2014). However, four other diploid lineages exist, spanning a wide range of habitats and latitude (Kolář et al. 2015, Kolář et al, unpubl. data). To fully understand the process of speciation in autotetraploids, we

need to assess the standing variation and the pattern of meiosis gene differentiation in all diploid lineages.

We will design (and use pre-designed) primers for the highly differentiated regions in the two meiotic genes ASY3 and SYN1, which were shown to be under strongest selection (Wright et al, 2014). As the different regions of the same gene are selected in different lineages, numerous primer combinations will be used for each gene. Amplicon sequencing will be performed to guarantee a high-throughput screening of the populations and the already produced RADseq genome-wide SNP data from the same populations will be used as indicator of a neutral background differentiation. The trade-off of this experimental approach is a cost-effective access to population-based data but not accounting for part of the novel variations in those genes.

We aim to answering following questions (1) whether the standing variation in diploids correlates with the lineages that gave rise to autopolyploids and (2) whether selection for meiosis vary across different habitats in diploids. In addition we will ask (3) if independent signals for selection exist when contrasting different diploid gene pools with distinct evolutionary histories.

## 2. RANGE-WIDE PATTERNS OF SELECTION: DOES EVOLUTION REPEAT ITSELF?

As stated above, preliminary results from RADSeq data suggest that autopolyploids originated three times independently during the evolution of the *A. arenosa* species complex. The age of each autopolyploid formation is different. In collaboration with the Bomblies Lab and the Yant Lab at Harvard University, we will use genome scan (shallow resequencing of the whole genome) data and test for selection signals in the putatively recently formed autopolyploid from Tristár Valley in the Western Carpathians and a putatively older, more established yet distinct autopolyploid lineage from the Southern Carpathians (Romania) and compare these data to the already genome-scanned, fully established autotetraploids from Western Europe (Hollister et al, 2012). We will compare each autotetraploid population with their geographically adjacent diploid population, for which we will also have genome-scans, and ask, (1) if the same genes evolve in the same way among different autopolyploid lineages, (2) can interploidal gene flow be detected, (3) adding the four remaining diploid population into genome-scan dataset I will also screen for the other functional categories of genes being under the selection in such putatively independent tetraploid lineages of *A. arenosa*.

## 3. MEIOSIS PAIRING STABILITY OF THE INDEPENDENT POLYPLOID LINEAGES

In this part, my aim is to compare meiosis phenotype, i.e. chromosome pairing and way of segregation in prophase 1, among autotetraploids with different levels of establishment. As stated above, we have evidence for a timely and spatially independent origin of autotetraploid lineages from diploids, and therefore I hypothesize that the level of proper (diploid-like) meiosis establishment is different among the three differently old lineages. To test this hypothesis, I want to look for meiosis phenotype of fully established autotetraploids from the Western Europe lineage (Arnold et al, 2015), of a local and putatively younger lineage from the Southern Carpathians (Romania) and of a single putatively recently formed autopolyploid population from Tristár Valley in the Western Carpathians. I will use colchicin-induced neo-tetraploids as a control. I expect the chromosomes to form multivalents and segregate irregularly in newly formed autotetraploids, while to form bivalents and diploid-like segregation in fully established populations. Testing this hypothesis and observing meiotic behavior has three main reasons: (1) study if there is any correlation between the degree of functional meiosis and the time passed since the polyploidization event. The results will complement my findings from task 2 (the genome scans). (2) I will screen pollen fertility through the alexander staining as another indication of the meiotic phenotype (3) I might question if another factors, apart from polyploidization, contribute to change and establishment of proper meiosis.

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