



Evolutionary and ecological role of apomixis and asexual reproduction

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ABSTRACT

In sexual organisms, meiotic recombination increases the likelihood evolution to progress faster by eliminating harmful mutations (the Hill-Robertson effect). Apomictic lineages (reproducing through asexual seeds), on the contrary, skip meiosis and do not undergo recombination; thus, they cannot restore adaptive alleles if deleterious mutations occur in these alleles. Recent population genetic studies have challenged the traditional view of apomixis as an evolutionary dead end and that it will lead to a loss of potential for adaptation to environmental changes because of the accumulation of harmful alleles and low variability. Fast phenotypic responses of apomicts to selection on short time scales indicate that apomixis imposes immediate reproductive isolation on the polyploid lineages, interferes with genetic cohesion forming fragmented gene pools into populations that develop independently, increases expansion range and ecological plasticity, and is associated with biodiversity, which indicates to accelerated genetic differentiation and ecological speciation in asexual complexes. It is suggested that apomixis may actually contribute to the diversification of angiosperms.

Keywords : Asexual reproduction, apomixis, apomeiosis, gene flow, ecological speciation, agamic complexes

Angiosperms reproduce mainly through sexual mode. Flowering plants produce seeds that are results from the fusion of haploid male and female gametes. Meiosis and fertilization as the main signs of sexuality secure the formation of genetically variable diploid progeny. Persistent genetic changes in populations due to meiotic recombination and a large number of mutations act as a source influencing the evolution of the population through acceleration or limitation of the rate of the population's adaptation. It was Darwin who believed that sexual reproduction is unprofitable, because the males can not produce offspring on their own (Darwin 1862a, 1876). By “cross-fertilization”, Darwin generally meant crossings between individual organisms, since he believed that effects of self-fertilization was similar to those of parthenogenesis. Those times Darwin was unaware of the existence of homo- and heterozygosity. However, he acknowledged that cross-fertilization has an immediate positive effect on the progeny, and thus justifies the energy costs of sexual reproduction as a sufficient advantage to balance the disadvantage associated with the production of males (Darwin 1862b). Later, the “Fisher-Muller” population-genetic theory also showed that sexual reproduction is very expensive and recognized the existence of sex in nature as paradoxical. At the same time, this theory focuses in particular on the advantages of sex due to the effect of a combination of useful mutations that are absent in asexual organisms (Muller 1932, Fisher 1999). Subsequently, several theories of the advantages of sexual reproduction over asexual were proposed. These theories can be divided into two different types: “mutational” theories (sexuality helps to get rid of

harmful mutations) and “ecological” theories (sexuality creates useful traits and variability, thereby produces material for selection and evolution).

However, the disadvantage of sexual reproduction is the segregation of beneficial traits in subsequent generations, so that the offspring can lose useful combinations of their parental genes (Spillane *et al.* 2004, Brukhin 2017). Crow and Kimura (1965) showed that, according to the Fisher-Muller model, sexual reproduction would be beneficial only under certain conditions, such as large population sizes and the relatively frequent occurrence of mutations. Perhaps most importantly, they demonstrated that in the case of co-adapted gene complexes, sex and recombination may be virtually unprofitable. This later became known as the “recombination cost”.

Recent studies and population genetic models of adaptation have challenged the traditional view of *de novo* mutations as the primary source of adaptive genetic variations, suggesting that pre-existing genetic and epigenetic variations can provide the best substrate for fast phenotypic responses to selection on short time scales (Messer *et al.* 2016). However, relatively low proportion of flowering plants propagate through asexual (apomictic) seeds and, thus, defies evolutionary theories. Such plants produce seeds after the formation of unreduced gametes that bypass meiosis, a mechanism called apomeiosis, and development of the embryo via parthenogenesis, independent of fertilization (Nogler 1984, Asker and Jerling 1992, Grossniklaus *et al.* 2001, Bicknell and Koltunow 2004, Van Dijk 2009, Kotani *et al.* 2014, Brukhin 2017). These apomictic plants produce

clonal progeny and dramatically affect existing structure of populations, reducing gene flow, genetic cohesion and variation. Thus, apomictic plants possess of reproductive and genomic mechanisms contrary to those well known in amphimictic (sexually reproducing) plants, they provide an excellent opportunity to test biologically important issues, such as the origin of meiosis, understanding the fundamentals of apomeiosis, seed formation, the role and importance of sexuality in maintaining and irradiation of the genetic changes, ecological adaptation, and creation of diversity. For example, under scenario of limited gene flow and loss of genetic cohesion, apomictic plants traditionally assumed to be doomed to extinction (Darlington 1939, Grant 1981). Nonetheless recent data have disproved this view and indicate that apomixis is associated with increased diversity (Hojsgaard *et al.* 2014a), suggesting that apomixis may actually contribute to the establishment of new polyploids (Hojsgaard 2018) and to the diversification of angiosperms. However, little is known about the processes and molecular background, confirming the diversity of plants in apomictic clades.

Incomprehensible phenomenon of the existence of asexual organisms—Despite the reproductive advantages, asexual lineages are rare among the multicellular taxa. It is expected that the absence of meiotic recombination will prevent the creation of genotypic variations persistently adaptive to new conditions and accelerate the stochastic accumulation of deleterious alleles, a phenomenon known as Muller's ratchet (Muller 1964, Charlesworth and Charlesworth 1997), leading to the genomic degradation and disappearance of the asexual accessions after a short existence. Even with these predictions of early extinction, known asexual lineages of animals and plants for which age estimates exist have lasted much longer than expected from the mathematical models derived theoretically (Lampert and Schartl 2008, Pellino *et al.* 2013). Obviously that asexuals have developed strategies to limit the negative effects of ameiotic reproduction, for example, diversity based on mutations and clonal competition (Charlesworth and Wright, 2001, Glémin *et al.* 2006, Ferreira de Carvalho *et al.* 2016, Warren *et al.* 2018). If rapid extinction is not a factor affecting the lifespan of asexuals, then why asexuals are so rare but not more common in nature? For a better understanding the nature of asexuality, it is necessary to identify the general mechanisms involved in the formation of various asexual organisms compared to standard sexual pathways. Perhaps the rarity of asexual lineages occurs due to the random probability of occurrence of genomic combinations necessary to avoid meiosis and create a functional hybrid genome, can apply to all living groups.

Evolution and development of ovules—The reasons for the sudden rise and superiority of angiosperms in the middle of the Cretaceous period are still a big mystery that was already noted by Charles Darwin. The evolution of the seed with its small female gametophyte (embryo sac), embedded in an

ovule tissues and fertilized by a greatly reduced male gametophyte (pollen grain), undoubtedly played a central role in the dissemination and predominance of spermatophytes (seed plants). Within this group, angiosperms are easily distinguished by their seeds, deeply enclosed in a nourishing and protective ovary, double fertilization, which initiates the formation of embryo and endosperm, and the presence of flowers that contain ovaries and stamens with anthers. The dramatic ecological and taxonomic diversification of angiosperms during the Cretaceous period due to expanse of free-sporing plants and other groups of seed plants (such as cycas, Cheirolepidaceae, and conifers) has been confirmed by various biological and ecological factors associated with environmental changes and an increase in leaf photosynthesis vigor (Boyce *et al.* 2009). However, the role played by various key reproductive innovations, such as pollination by insects, fruit adaptations, and others that may had led angiosperms to ecological dominance ~ 30 million years ago after taxonomic diversification, is still unclear (Wing and Boucher 1998, Hu *et al.* 2008). In any case, developmental advancement and reproductive plasticity, closely related to the origin of the angiosperm flowers, played a central role in the irradiation of angiosperms (Friedman 2006, Endress 2011, Barrett 2013). In the past studies the sexual events of sporogenesis, gametogenesis and fertilization in angiosperms were considered as a predominant source for adaptation and evolution. The nature and potential role of the atypical reproductive patterns, alternative to the sexual egg cell and seed development, such as formation of the asexual seeds via apomixis (Asker and Jerling 1992), had not been evaluated in an evolutionary perspective. Apomictic plants are characterized by specific abnormalities in normal sporogenesis, gametogenesis, and fertilization events during seed formation, the hallmarks of apomixis are i) apomeiosis ii) parthenogenesis iii) development of functional endosperm by autonomous or pseudogamous way, which is characterized by a distorted ratio of embryo to endosperm ploidy (Asker and Jerling 1992, Naumova *et al.* 2001, Koltunow and Grossniklaus 2003, Aliyu *et al.* 2010). Apomictic plants are found at low frequencies but are scattered over many angiosperm families and are associated with highly diversified lineages (Hojsgaard *et al.* 2014b), confirming the hypothesis that apomixis can serve as a springboard for expanding the range and diversifying plants (Hojsgaard and Hoerandl 2015). Analysis of the phylogenetic relationship between sexual and apomictic development can help to understand the extent to which such a rare reproductive strategy could contribute to the past or present evolutionary success of angiosperms (Charlesworth and Wright 2001, Glémin *et al.* 2006, Rushworth *et al.* 2011, Lee and Mitchell-Olds 2011, Lee *et al.* 2017).

The evolution of land plants passed through an impressive specialization and reduction of reproductive structures. In seed plants (that is, gymnosperms and

angiosperms), such processes are clearly manifested in the evolution of the female gametophytes (megagametophytes and/or embryo sacs) and structures that produce functional seeds. Throughout the entire phylogenetic tree of angiosperms embryo sacs show a wide range of developmental patterns, including changes in cell division, polarization, cellularization time, etc. (Hofmeister 1847, Geerts 1908, Modilewski 1909, Palm 1915, Maheshwari 1948, 1950, Battaglia 1989, Johri *et al.* 1992, Brukhin *et al.* 2005, Friedman 2006, Endress 2011, Bachelier and Friedman 2013). Often the taxonomic complexity is associated with a gradual decrease in the number and location of the nuclei and cells that form the mature embryo sac. While in gymnosperms, the female gametophyte (called megagametophyte that also serves as a haploid endosperm) is much larger than that of angiosperms, consisting of several hundreds or even thousands of cells with several archegonia enclosed within jacket cells (Maheshwari 1950), in the female gametophytes (embryo sacs) of more specialized angiosperm species such as *Oenothera spp.* (Geerts 1908, Modilewski 1909, Maheshwari 1950), *Schisandra spp.* or *Panicum spp.* (Battaglia 1989) only 4 meiotically reduced nuclei are formed, and one cell (central cell) initiates after fertilization development of the triploid endosperm, a nutritional tissue risen from two parents. In *Plumbagella* embryo sac consists of two haploid micropylar nuclei and two triploid chalazal nuclei. The haploid nucleus nearest to the micropylar end becomes organized into the egg cell. The remaining two nuclei fuse to form a tetraploid secondary endosperm nucleus and no synergid cells are produced (Fagerlind 1938, Brukhin *et al.* 2005). New findings in some species of most basal angiosperms, e.g. *Nufar* showed that their embryo sacs have four nuclei, four cells, and develop a diploid endosperm (Williams and Friedman 2002, Friedman and Williams 2003, 2004). The *Polygonum*-type monosporic female gametophyte, which produces an 8-nucleate 7-celled embryo sac is the most common among the over 70% of angiosperms.

Despite the extraordinary simplification of embryo sac in course of the modern angiosperms' evolution, it is a complex and highly specialized system responsible for important for evolution events such as changing the ploidy and further, after fertilization, maintaining the ratio of maternal to paternal genomes (2m:1p) that is required for the development of functional endosperm (Grossniklaus 2001, Curtis and Grossniklaus 2008). Although being crucial for seed formation, this requirement for endosperm development might be mitigated in the apomictic relaxed dosage constrain programs, which develop unreduced female gametophytes and form asexual seeds with distorted ratios of the parental genomes as 1m:1p, 4m:3p, 8m:1p, 3m:12p etc. (Grossniklaus 2001, Naumova *et al.* 2001, Aliyu *et al.* 2010, Hojsgaard and Hoerandl 2015), the programs that allow endosperm development, protect reproductive system and facilitate plant

species survival under transition to polyploidy. The apomeiotic embryo sacs unlike sexual embryo sacs have unreduced nuclei and mainly represented by the four-celled, four nuclear (*Panicum*-type) or seven-celled, eight nuclear (*Hieracium*-type) embryo sacs (Johri *et al.* 1992, Asker and Jerling 1992, Brukhin 2017), although deviations from these patterns have also been described, for example, *Paspalum*-type embryo sacs that consists of five unreduced nuclei and four cells (Hojsgaard *et al.* 2008). The wide-ranging researches of the occurrence and ecological behavior of apomict and sexual plants among the large lineages of angiosperms were shown in a range of remarkable studies (Battaglia 1989, Carman 1997, Lee and Mitchell-Olds 2011, Hojsgaard *et al.* 2014b, Lovell and McKay 2015, Lee *et al.* 2017, Lovell *et al.* 2014, 2017 and others) where comprehensive analyses of the phylogenetic and evolution relationships, as well as genetic interaction between the apomictic and meiotic lineages of angiosperms in agamic complexes were reported.

The role of apomixis in ecological adaptation—As already mentioned above, apomixis in angiosperms produces genetically homogeneous progeny (Nogler 1984, Asker and Jerling 1992, Grossniklaus *et al.* 2001, Bicknell and Koltunow 2004, van Dijk 2009, Kotani *et al.* 2014, Brukhin 2017). Its importance for the evolution and biodiversity of angiosperms is difficult to assess mainly because of insufficiency of apomictic research and documentation data for many taxonomic groups. Currently apomixis has been identified in hundreds of plant species belonging to over 400 families (Nogler 1984, Carman 1997, Hojsgaard *et al.* 2014b). There have been carried out a range of studies identifying mutants in model plants displaying components of apomixis, or by isolating the relevant genes from an apomictic species (e.g. Grossniklaus 2001, Rodriguez-Leal and Vielle-Calzada 2012, Pupilli and Barcaccia 2012, Barcaccia and Albertini 2013, Conner and Ozias-Akins 2017). Three apomictic genera that have been studied in depth were *Hieracium*, *Paspalum* and *Pennisetum* (Asker and Jerling 1992, Carman, 1997, Hojsgaard *et al.* 2014b), research in these natural apomicts have greatly contributed to progress in the field (reviewed in e.g. Ortiz *et al.* 2013, Bicknell *et al.* 2016, Conner and Ozias-Akins 2017). Also a great deal of research were devoted to study the genetic regulation of apomixis, ecology and interaction of sexual and asexual accessions within the agamic complexes of the plants from the *Boechera* species (Böcher 1951, Sharbel and Mitchell-Olds 2001, Dobes *et al.* 2004, Schranz *et al.* 2005, Sharbel *et al.* 2005, Windham and Al-Shehbaz 2006, 2007a, b, Voigt-Zielinski *et al.* 2012, Lovell *et al.* 2014, 2017, Mandakova *et al.* 2015, Schilling *et al.* 2018). There are many different immediate causes of asexuality. With regard to angiosperms, recent genetic analysis has shown the existence of chromosomally transmitted determinants for asexuality (Matzk *et al.* 2000). Some of them look like

“supergens,” that is, discrete chromosomal regions with a number of genes and functions in close connection (Grossniklaus *et al.* 2001). Nuclear factors can interact with the level of ploidy, which also makes this factor the cause of asexuality. As discussed earlier, hybridity itself can cause asexuality due to the conflicting signaling it produces. In plants of the *Boecheira holboelli*, sensu lato complex the cause of asexuality is believed could be *Del* and *Het'* chromosomes (Mandakova *et al.* 2015) and / or the *APOLLO* locus (Corral *et al.* 2013). *APOLLO* (Aspartate Glutamate Aspartate Aspartate histidine exonuclease) is one of the important genes associated with apomixis in *Boecheira*. The *APOLLO* locus was shown to have several polymorphic alleles associated with apomixis. All studied apomictic plants contained at least one of the apo-alleles, while both copies in the sexual

genotypes were sex-alleles. Kliver and coauthors (2018) studied this locus in detail in species of the *Boecheira* genus and in other species of Brassicaceae. Along with an exact copy of the *APOLLO* locus, two other, more distant copies were found that may indicate to the past duplication events. Phylogenetic tree reconstruction based on the orthologs of this gene in other species (Fig. 1) showed that all the Brassicaceae genomes taken for the study also carried the three copies associated with clusters of orthologous genes. The study of apo- and sex-alleles *APOLLO* showed that these alleles arose after the separation of the genus *Boecheira*, and make up two separate clades. The authors also showed that the branch leading to apo-alleles is under the positive selection, which is typical for paralogues that are required to serve a novel function (Kliver *et al.* 2018).

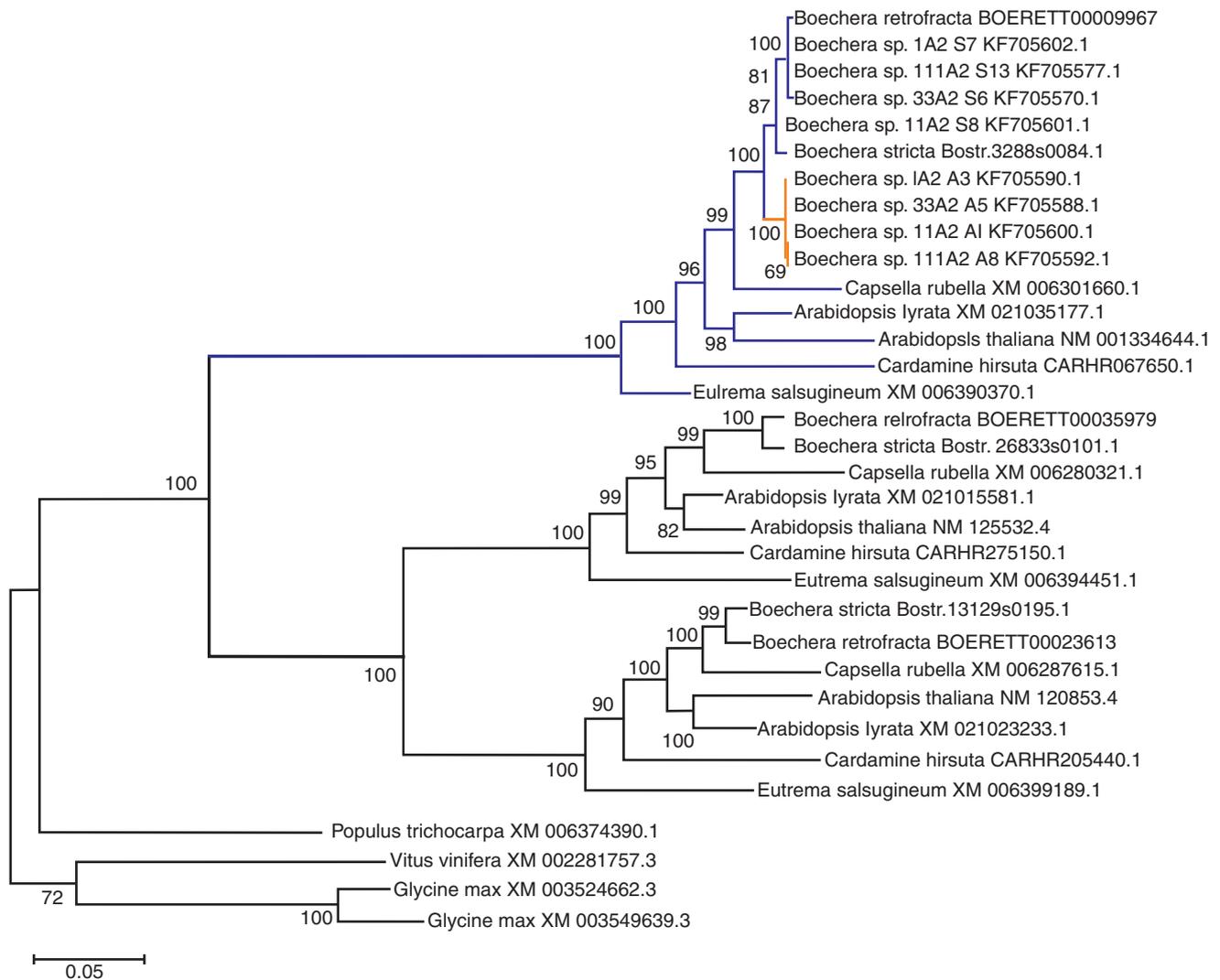


Fig. 1– Phylogenetic tree of the isoforms of *APOLLO* locus (exonuclease NEN) in seven species of interest and alleles of *APOLLO* locus of apomictic *Boecheira* species from Corral *et al.* (2013). Sequences of *Populus trichocarpa*, *Vitus vinifera* and *Glycine max* were used as outgroup. The clade related to the *APOLLO* locus is shown in blue with apo-alleles shown in red. Numbers near nodes represent corresponding bootstrap support. (adopted from Kliver *et al.* 2018).

Traditionally, based on the assumption that the loss of genotype heterogeneity in apomictic populations will lead to a loss of potential for adaptation to environmental changes, apomixis was considered as an evolutionary dead end (Darlington 1939, Stebbins 1950, Grant 1981). It was believed that the apomictic lineages were doomed to extinction. Later studies based on the empirical observations changed this view to consider apomictic lineages as closed systems, revealing that they carry significant genetic variation and represent dynamic and flexible systems (Khokhlov 1946, Carman 1997, Hoerandl and Hojsgaard 2012). The Russian geneticist Sergey Khokhlov assessed and reconsidered the accumulated by that time information relating to the various aspects of this phenomenon, he expressed and substantiated an absolutely new point of view at the evolutionary role of apomixis. His conclusion was that apomixis is a natural step in the evolution of higher plants, due to the development of the evolutionary tendency toward the reduction of the gametophyte, which should end with its final disappearance. Khokhlov concluded that apomixis is a progressive phenomenon that leads to the improvement of the angiosperm reproduction system and to the transition to a new type of plants, i.e. agamosperous plants. This view was somewhat radical, it greatly diminished the role of sexual reproduction, but the views of the scientist emphasized the crucial role of apomixis in the evolution and strengthening of the phenotypic plasticity of reproductive systems (Khokhlov 1946).

There is a well-known positive correlation between polyploidy and asexuality (Suomalainen *et al.* 1987, Asker and Jerling 1992), which is easy to understand from many points of view, but not from all. Meiosis often fails in polyploids, especially in those that have an odd number of genomes, due to difficulties in connecting more than two chromosomes of each species. Therefore, it is not surprising that asexual reproduction is a great advantage for polyploids - without it, many polyploids would not be able to reproduce and would never have been seen in nature. Another explanation for the association between asexuality and polyploidy is that a complex genotype in polyploids can signal meiosis and gamete control points in such a disturbed but precise way that subsequent formation of unreduced gametes and spontaneous embryogenesis naturally follow (Bengtsson, 2009). It is expected that various evolutionary forces will drive the evolution of lineages that differ in their reproductive modes. In sexuals, recombination increases the likelihood of evolution to progress faster by eliminating harmful mutations (the Hill-Robertson effect) (Hill and Robertson 1966, Felsenstein 1976). Apomictic lineages, on the contrary, reproduce asexually and do not undergo recombination; thus, they cannot restore adaptive alleles if deleterious mutations occur in these alleles (Charlesworth 2008). Consequently, one can expect accumulation of harmful alleles, a phenomenon

known as the Muller ratchet (Muller 1964, Charlesworth and Charlesworth 1997). Recent comparisons of apomictic and sexual lineages in *Boecheera spp.* supported these population genetic expectations (Lovell *et al.* 2013, 2014, 2017). Lovell and colleagues (2017) used the apomictic and sexual populations of *B. spatifolia* to study patterns in nucleotide changes in the both reproductive regimes by whole genome sequencing. They found an increased sequences diversity and heterozygosity along with an increased accumulation of mutations in apomictic populations (Lovell *et al.* 2017). Similarly, in a broader study of 37 natural populations of four *Boecheera* species (*B. stricta*, *B. retrofracta*, *B. polyantha* and *B. pendulocarpa*) microsatellite markers showed the same trend (Lovell *et al.* 2013): higher levels of genome heterozygosity were found in apomicts compared with sexuals, regardless of the ploidy level of the apomictic plants.

A literature analysis helps to reveal some important facts for understanding the role of apomixis in the evolution of angiosperms (Hoerandl and Hojsgaard 2012, Hojsgaard *et al.* 2014b). For example, unlike previous assumptions about predisposition to the development of apomixis within large families (for example, Poaceae, Asteraceae and Rosaceae) (Richards 1997, Ozias-Akins and van Dijk 2007), apomixis was found to be taxonomically widespread without a clear trend towards certain groups and occurs along with sexuality at all taxonomic levels (Hoerandl and Hojsgaard 2012, Hojsgaard *et al.* 2014b). Such phylogenetic scattering is closely related to biodiversity measurements. The number of apomict containing genera positively correlates with the total number of genera per subfamily, family, and level of order (Hojsgaard *et al.* 2014b). In general, the apomict-containing groups were larger, in other words, they had a larger number of families or genera than the non-apomictic groups. Moreover, many genera containing apomicts turned out to be more cosmopolitan. According to Hojsgaard and co-authors (2014b), apomictic groups are significant among very diverse geographically-extensive taxa, from genera to orders, and the observations that transitions from apomixis to sex occur in several taxa confirm the opinion that apomixis can work as a springboard for diversification. Since the transition from sexual reproduction to apomixis can fix adapted useful gene combinations and genotypes and thereby facilitate the expansion of the species, while reversal to sexuality will use hidden variability and increase genetic and genomic heterogeneity within-plant, in sexual-apomictic groups the range of expansion and diversification at the species and genus levels may occur faster than in exclusively sexual systems (Hojsgaard *et al.* 2014b, Hojsgaard and Hoerandl 2015).

However, not easy to understand why the well-studied phenomenon of apomixis, which is used here to denote asexual reproduction through seeds in angiosperms, is never

found in diploids or, at least, very rarely. After some candidates were excluded earlier by strict genetic tests, today it is assumed that only one genus produces natural diploid apomicts, namely the already mentioned *Boechera* genus (a close relative to the well-studied model plant *Arabidopsis thaliana*). The genomes of the apomictic lineages of *Boechera* are characterized by extremely high heterozygosity, accompanied by alloploidy and aneuploidy, which results from the interhybridization events (Koch *et al.* 2003, Schranz *et al.* 2005, Mandakova *et al.* 2015, Lovell *et al.* 2017, Kliver *et al.* 2018). The reason of lack of apomixis among diploid plants is probably due to the complex reproductive mechanisms used by angiosperms and a special nature of the factors causing apomixis, but not by asexuality itself (as discussed in Grossniklaus *et al.* 2001), since diploid apomicts can be obtained experimentally (Nogler 1984, D'Erfurth *et al.* 2009). The existence of many diploid asexual animals also suggests that there are no strong evolutionary obstacles that may hinder the functioning of asexuality at the diploid level. In the apomictic lineages, evolution occurs both as a result of genetic drift and natural selection (Charlesworth and Wright 2001, Glémin *et al.* 2006). A selection efficiency, as we already discussed, would be higher in sexual lineages, which undergo recombination, and lower in apomicts, which, in turn, would increase the risk of extinction due to the accumulation of deleterious alleles and inability to adapt to the environmental changes (Darlington 1958, Muller 1964, Bengtsson 2009). Contrary to these expectations, the *Boechera* genus is very diverse, including a wide range of apomictic accessions (Böcher 1951, Sharbel and Mitchell-Olds 2001, Dobeš *et al.* 2004, Schranz *et al.* 2005, Sharbel *et al.* 2005, Windham and Al-Shehbaz 2006, 2007a, b, Voigt-Zielinski *et al.* 2012, Alexander *et al.* 2013, Lovell *et al.* 2014, 2017). The possible explanation for the survival of *Boechera* apomictic lineages could be the intra- and inter-species gene flow within the genus (Böcher 1951, Sharbel and Mitchell-Olds 2001, Dobeš *et al.* 2004a, b, Schranz *et al.* 2005, Beck *et al.* 2011, Lovell *et al.* 2013, 2017, Li *et al.* 2017, Schilling *et al.* 2018). The gene flow mainly occurs from the sexual lineages to apomicts, while apomicts are capable to produce a small amount of pollen, that can pollinate the sexual plants and carry the dominant factor(s), which gives to apomicts a selective advantage. The mutual gene flow between apomictic and sexual individuals may allow the introgression of adaptive alleles from sexual to apomictic lineages, as was established by Van Dijk and colleagues (2009) for *Taraxacum spp.* In addition, apomixis in *Boechera spp.* is facultative when different individuals can produce both sexual and apomictic progeny (Schranz *et al.* 2005, Aliyu *et al.* 2010). These probable cases of sexuality in apomicts might be sufficient to purge deleterious mutations and establish the fitness of the

apomictic lineages, ensuring their evolutionary survival (Van Dijk *et al.* 2009).

The evolutionary genomics and speciation in asexual plants—The central process of evolution is the formation of new species (speciation). Biodiversity and increase the evolutionary complexity of organic life on Earth took place through speciation. Speciation is considered to be a continuous process of gradual genetic, physiological, and morphological changes that lead to population divergence and the emergence of reproductive isolation mechanisms (Shaw and Mullen 2014, Seehausen *et al.* 2014). Thus, understanding the sources and distribution of permanent genetic, cytological, and morphological changes is crucial for identifying the evolutionary processes leading to speciation. According to Levin (2000), we can distinguish four stages in the life or history of species: birth or origin, expansion, differentiation and loss of interaction, and decline and extinction (Levin 2000). While we have a variety of information for the origin and expansion of species, derived from phylogeographic studies, direct information about the evolutionary history of species is insufficient, simply because we are limited to species that have evolved recently.

The emergence of the next generation sequencing techniques had provided opportunities to study general genomic changes associated with speciation at the individual and population levels, and gave unprecedented resolution of the genetic diversity of species and their evolutionary history (Morris and Shaw 2018). This made it possible to identify incipient species (for example, between sympatric / parapatric populations), revealing details about the divergence of species in the initial stages (Marques *et al.* 2016, 2018). The extent to which growing species are subjected to geographical differentiation depends on the gene pools of species, breeding systems, distribution patterns, demographic properties, and ecological chances (Levin 2000). In expanding species, local adaptation plays a central role promoting differentiation of populations and reproductive isolation (Richardson *et al.* 2015). The emergence of reproductive barriers that maintain a genetic and phenotypic signatures of populations in geographic proximity can be triggered by either divergent selection (i.e., “environmental”), which creates some external reproductive barriers, or evolution of genetic incompatibility (for example, genetic drift or genomic conflict) that cause intrinsic reproductive barriers (Seehausen *et al.* 2014). The theory of ecological speciation suggests that genetic differentiation across the genome will be heterogeneous (Nosil 2012). During genomic differentiation and speciation with-gene-flow loci under destructive environmental selection that can provide local adaptation and extrinsic (post-zygotic) reproductive isolation, or loci conferring intrinsic (pre-zygotic) reproductive isolation, will be more resistant to

gene flow than the rest of the genome and lead to increased differentiation around them (Wu 2001, Marquis 2016). These genome regions that are resistant to gene flow are collectively referred to as “genomic islands of differentiation” (Turner *et al.* 2005, Harr 2006, Aeschbacher *et al.* 2017) and are considered points around which reproductive isolation crystallizes. Speciation begins with several islands of differentiation in genome. With increase in reproductive isolation (via linkage of selected loci or pleiotropy with other reproductive traits), the number of islands is estimated to grow, and the rest of the genome will begin to diverge due to background selection, selection not related to speciation and due to drift (Nosil 2012, Via 2012, Marques *et al.* 2016, 2018). Consistent with the emerging ecological speciation, Marques and colleagues (2016) found islands of significant genomic differentiation, opposing the flow of genes between ecotypes in both sympatry and parapatry, probably due to divergent selection among habitats. This suggests that adaptive genomic differentiation may occur and persist in sympatry at very early stages of ecotype divergence and that the genomic adaptation architecture may contribute to this (Marques *et al.* 2016, Aeschbacher *et al.* 2017). The findings suggest that adaptation does occur along with gene flow, and emphasize the interaction between gene flow, the structure of the genome and natural selection in determining the genetic architecture of adaptation. Since gene flow and selection interact, contributing to adaptive divergence in areas with low recombination (Samuk *et al.* 2017), these interactions can be expected to be weakened by the imposed absence of gene flow and recombination, contributing to genetic divergence throughout the genome. The existence of factors that dramatically change local and regional gene flow rates, such as polyploidy or asexuality, make it possible to assess the dynamics of individual and population genomic differentiation and adaptive divergence.

Polyploidy is one of the most significant evolutionary forces in flowering plants. It is estimated that about 30–80% of angiosperms are polyploid (Jiao *et al.* 2011). Polyploidy contributes not only to diversification through genome duplication, but also by fragmentation of gene pool (Otto 2007; Soltis *et al.* 2009; Cheng *et al.* 2018). Once a polyploid emerged, it acquires partial reproductive isolation from parental diploids, mainly due to the effects of the triploid block and ploidy barriers (Koehler *et al.* 2010, Hojsgaard 2018). Consequently, diploid parental and polyploid derivatives remain connected by low and recurrent exchange of genetic material, while their respective gene pools retain a certain level of genetic cohesion (de Wet and Harlan 1970, Grant 1981, Hollister *et al.* 2012). These cytotype associations, called polyploid complexes, are strongly influenced by their genetic systems (i.e., reproductive regimes, reproduction

modes, life cycle, etc.), which determine genetic variability and population structure and niche evolution between different levels of ploidy (Doyle *et al.* 1999, Hollister *et al.* 2012, Mau *et al.* 2015, Karunaratne *et al.* 2018). Therefore, the evolutionary pathways that new polyploids follow can occur independently of parental diploids (Muenzbergova *et al.* 2013), which means that polyploid complexes constantly undergo a divergent evolution immediately after their formation, which to some extent counteracts by the level of gene flow and interploidy genetic cohesion.

It is now well known that polyploidy and / or hybridization alter the genomic composition, gene expression, and epigenetic regulation of phenotypic traits (Comai 2005, Chen 2007, Cifuentes *et al.* 2010, Grandont *et al.* 2013). This situation, called “genomic shock,” contributes to the trait divergence and is often associated with changes in the reproductive system, such as self-fertilization (autogamy) or apomixis (asexual seeds) (Comai 2005, Hojsgaard *et al.* 2013). Changes in the nature of the breeding system are of particular interest because they affect the number and distribution of genetic variations within species (Igic *et al.* 2006, Anderson *et al.* 2011). Both self-fertility and apomixis are mechanisms that allow uniparental reproduction (i.e. reproduction through a single parent), which is a favorable condition for colonization of the population where pollinators or partners for mating are limited (Baker's law; Baker 1955, 1967) fostering the occurrence of specific geographical distribution mode between sexuals and apomicts (geographical parthenogenesis) (Bierzuchudek 1985, Hoerandl 2006). Thus, uniparental reproduction promotes the founder's effect and creates bottlenecks in population, which is well suited for research on the rapid evolution to novel conditions (Anderson *et al.* 2011).

It is known that apomictic plants constrain recombination, skipping meiosis and syngamy during seed formation (Asker and Jerling 1992), thereby producing clonal lineages. In such lineages, since meiosis is not completely eliminated and low levels of residual sexuality are common, recombinant individuals can be sporadically formed (Cosendai *et al.* 2011). However, the repeated lack of recombination provides accumulation of mutations throughout the genome, and therefore it is expected that apomict polyploid clones will show accelerated genetic divergence.

Apomixis drastically influences both biological (i.e. gene flow, purifying mutations) and geographic (i.e. dispersion) features that simultaneously act to limit genetic cohesion and facilitate to range expansion, thereby endorsing synergistic ecological differentiation. Consequently, polyploid / allopolyploid / aneuploid complexes with apomixis, which restrict gene flow to diploid sexual parents, represent an ideal system for

assessing genetic differentiation within and between individuals and how the interaction between the (reduced) gene flow, genome structure, and natural selection determines the genetic architecture of adaptation and the resulting speciation among populations of apomictic polyploid complexes. The effects of low gene flow on population structure and genetic separation have been documented for a small number of apomictic-sexual systems using anonymous markers (e.g. AFLP) (for example, *Ranunculus sp.*, Cosendai *et al.* 2011, 2013, *Hypericum sp.*, Koch *et al.* 2013,

To date there is available a very little information about genomic differentiation among populations of *Paspalum sp.*, Karunaratne *et al.* etc.). Allopatric apomictic polyploids show lower levels of variability than those who live in sympathy with sexual diploids (agamic complexes) (Daurelio *et al.* 2004, Symonds *et al.* 2010, Havananda *et al.* 2011). Closely related to their life history, apomictic lineages often show similar levels of variability compared to sexual parents, but always demonstrate a greatly reduced genotypic diversity (Paun *et al.* 2006, Cosendai *et al.* 2013, Dias *et al.* 2018). Even if asexual reproduction is expected to weaken the adaptive capacity to new environmental conditions (Comai 2005), observations show that apomixis combined with polyploidy contributes to rapid adaptation to new environments. This is likely to occur via multiplication of highly fitted genotypes exhibiting wide tolerance to ecological conditions that exhibit better physiological abilities to use natural resources, whether through broader ecological tolerance compared to sexuality or through the separation of the sexual niche by fixing a subset of their genetic changes (Vrijenhoek 1984). However, molecular evidence of this is still inconclusive, mainly due to the lack of sequence level information.

The evolutionary view that apomictic plants have a reduced adaptation potential and therefore are not viable has changed in recent years as we have a clearer view of the potential role of apomixis supporting plant diversification (Hojsgaard and Hoerandl 2015, Hojsgaard 2018). According to Carman (1997) and Hoerandl and Hojsgaard (2012), apomicts represent a transitional stage in the origin and evolution of polyploid complexes, necessary for the stabilization of genetically and chromosomal-time unbalanced polyploid genotype, and a springboard for diversification. Using the combined advantages of apomixis and polyploidy (uniparental reproduction, clonal reproduction and higher ecological plasticity), highly efficient polyploid genotypes could play a role of temporary pioneers of new, previously unexplored ecological niches that expand the geographical distribution of diploid parent cytotypes. Limited gene flow and reduced efficacy to purge mutation would contribute to genetic divergence and the survival of locally adapted genotypes. Following changes in sexual cytotypes (Chapman *et al.* 2003, Ortiz *et al.* 2013) will contribute to

further genetic divergence and allopatric speciation (Hoerandl and Hojsgaard 2012). This model is confirmed by the analysis of sister clades for the presence or absence of apomixis in angiosperm phylogenesis, which shows that apomixis is largely associated with families of a greater number of genera and a higher number of species (Hojsgaard *et al.* 2014a).

CONCLUSION

Summarizing all said above, apomixis 1) imposes immediate reproductive isolation on the polyploid lineages, 2) interferes with genetic cohesion forming fragmented gene pools into populations that develop independently, 3) accelerates the accumulation of mutations, 4) increases expansion range and ecological plasticity in combination with polyploidy, and 5) is associated with biodiversity, which indicates to accelerated genetic differentiation and ecological speciation in asexual polyploid complexes. However, molecular evidence of how speciation can occur within asexual origin and whether it will be successful at all is still missing. To get an idea of the general mechanisms of speciation and the evolutionary mystery of the relative success of sexual and asexual reproduction, one can use the high-throughput sequencing methods and high-quality transcriptome analysis of plants growing in agamic complexes, such as those from the genus *Boechera*, that satisfy the above points to determine the relative importance of geographic distances and habitat variables in structuring asexual populations, understanding how genetic and ecological differentiation develops in sexual-asexual taxa, and help to clarify some of the evolutionary questions mentioned above. This may shed light on the mechanisms of speciation and help to uncover the evolutionary puzzle of the relative success of sexual and asexual reproduction, as well as help to provide detailed information on genetic differentiation within and between cytotypes and populations. The latter may be important for answering evolutionary questions of how the determination of the molecular basis for differentiation between cytotype niches, their reproductive interactions, gene flow, genome structure, and their natural selection determine the genetic architecture of adaptation to various environmental conditions.

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