*Annals of Botany* **XX**: 1–18, 2024
https://doi.org/10.1093/aob/mcae044, available online at www.academic.oup.com/aob

ANNALS OF BOTANY
Founded 1887

# Apomixis and the paradox of sex in plants

**Elvira Hörandl***

*Department of Systematics, Biodiversity and Evolution of Plants (with herbarium), University of Göttingen, Göttingen, Germany*
**For correspondence. E-mail ehoeran@uni-goettingen.de*

Received: 11 December 2023 Returned for revision: 7 March 2024 Editorial decision: 11 March 2024 Accepted: 15 March 2024

- **Background** The predominance of sex in eukaryotes, despite the high costs of meiosis and mating, remains an evolutionary enigma. Many theories have been proposed, none of them being conclusive on its own, and they are, in part, not well applicable to land plants. Sexual reproduction is obligate in embryophytes for the great majority of species.
- **Scope** This review compares the main forms of sexual and asexual reproduction in ferns and angiosperms, based on the generation cycling of sporophyte and gametophyte (leaving vegetative propagation aside). The benefits of sexual reproduction for maintenance of genomic integrity in comparison to asexuality are discussed in the light of developmental, evolutionary, genetic and phylogenetic studies.
- **Conclusions** Asexual reproduction represents modifications of the sexual pathway, with various forms of facultative sexuality. For sexual land plants, meiosis provides direct DNA repair mechanisms for oxidative damage in reproductive tissues. The ploidy alternations of meiosis–syngamy cycles and prolonged multicellular stages in the haploid phase in the gametophytes provide a high efficiency of purifying selection against recessive deleterious mutations. Asexual lineages might buffer effects of such mutations via polyploidy and can purge the mutational load via facultative sexuality. The role of organelle–nuclear genome compatibility for maintenance of genome integrity is not well understood. In plants in general, the costs of mating are low because of predominant hermaphroditism. Phylogenetic patterns in the archaeplastid clade suggest that high frequencies of sexuality in land plants are concomitant with a stepwise increase of intrinsic and extrinsic stress factors. Furthermore, expansion of genome size in land plants would increase the potential mutational load. Sexual reproduction appears to be essential for keeping long-term genomic integrity, and only rare combinations of extrinsic and intrinsic factors allow for shifts to asexuality.

**Key words:** Apomixis, apogamy, polyploidy, meiosis, Muller's ratchet, haploid selection, stress response, terrestrialization.

## INTRODUCTION

The predominance of obligate sexual reproduction in eukaryotes, specifically observed in plants and animals, is one of the outstanding questions of evolutionary biology. Sex is defined here in a broad sense as 'a process in which the genomes of two parents are brought together in a common cytoplasm to produce progeny, which may then contain re-assorted portions of the parental genomes' (Birdsell and Wills, 2003). Sexual reproduction in eukaryotes implies manifold costs, which can be grouped into the costs of meiosis and the costs of mating. Meiotic recombination can potentially break up favourable gene combinations, thereby resulting in less adapted gene combinations and reduced fitness in the offspring (Otto, 2009). The cellular–cytological process of meiosis itself is risky, time-consuming and prone to errors (Lewis, 1987). Homologue pairing at meiosis requires a tight control over the whole chromosome to avoid ectopic or non-allelic recombination between chromosomes, which would have deleterious effects (Sepsi and Schwarzacher, 2020). Mating requires two parents to produce offspring, and they have to come into contact for the fertilization process. These activities confer costs of mate finding, mate attraction and potential risk of exposure to predators or herbivores during these activities, among others. The success of mating also depends on a minimum density of a population to bring the gametes of two parental individuals together. If parents exhibit separate sexes and only females produce offspring, as in most animals, then there is a high cost of having male individuals ('cost of males'; Smith and Maynard-Smith, 1978). Separation of sexes also implies the costs of genome dilution: a sexual oogamous parent transmits only 50 % of its genes to the offspring but invests 100 % of nutritious and cytoplasmatic resources into the zygote. In comparison to an isogamous organism, it suffers a 50 % cost in the efficiency of reproduction. In hermaphrodites these costs are reduced, but there is still a cost of producing male and female gametes. However, plants, as sessile organisms, have a cost of gamete transfer: in wind-pollinated plants it requires mass production of pollen, and in animal-pollinated plants there is the cost to produce floral displays to attract pollinators. However, these costs apply to the sporophyte and are not sex specific. It is difficult to provide evidence for general selective advantages of sexuality to compensate for these costs. About 20 hypotheses exist for explaining the paradox of sex (Birdsell and Wills, 2003), and they can be grouped into three main concepts: (1) sexual reproduction results in genetic recombination and higher variation in the offspring, upon which selection can act more efficiently, dating back to August Weismann in the 19th century (Burt, 2000); (2) sexual reproduction is a comprehensive DNA restoration tool to maintain integrity of the genome over generations (Muller, 1964; Kondrashov, 1988; Hörandl, 2009); and (3) sexual reproduction has been established in ancestors of eukaryotes, and various constraints evolved to shift from sexual to asexual reproduction (Williams, 1975; Margulis and Sagan, 1986; Engelstädter, 2008; Hörandl, 2009). These concepts are not mutually exclusive, and various combinational theories have been proposed.

A further difficulty for understanding the benefits of sexuality arises from the huge variety of reproductive pathways and sexual systems in the different kingdoms of eukaryotes, with many different forms of asexual reproduction (reviewed by Hörandl *et al.*, 2020). Moreover, costs and benefits of sex are not distributed evenly among eukaryotes (Lewis, 1987). Most evolutionary hypotheses around the paradox of sex were developed from examples in the animal kingdom, whereas other clades were often not considered. Asexual reproduction appears in eukaryotes in many different developmental pathways on the tips of the phylogenies (Simon *et al.*, 2003; Hojsgaard *et al.*, 2014*b*), but often results in abundant and widespread taxa (Bierzychudek, 1985; Kearney, 2005; Hörandl, 2023). However, in both animals and plants <1 % of species reproduce asexually, whereas in other eukaryotic clades, e.g. in fungi and protists, asexuality is thought to be more common (Burt, 2000). This raises the question of whether universal selective pressures do exist for maintenance of sex and whether universal theories can be developed to explain the predominance of sex.

Land plants are of special interest for this question because, on the one hand, sexual reproduction is predominant, similar to animals (Mogie, 1992); on the other hand, some theories developed for animals are not readily applicable to plants because autotrophic organisms have different physiological constraints (Hörandl and Hadacek, 2020). In this review, I will first briefly review the forms of sexual and asexual reproduction in land plants; comprehensive reviews have been presented elsewhere (Asker and Jerling, 1992; Mogie, 1992; Hojsgaard *et al.*, 2014*b*; Grusz, 2016; Hojsgaard and Pullaiah, 2023). Based on the main developmental pathways, I will discuss costs and benefits of meiosis–mixis cycles as the core process of sexuality for land plants and expand on the relevance of organelle–cytoplasm compatibility for successful reproduction. For land plants, plastome–nuclear interactions are of specific interest. Then I will briefly review costs and benefits of separate vs. combined sexes in plants and review the potential constraints of shifts from sex to asexuality. I will suggest a theory within a phylogenetic framework of Archaeplastida, which does not regard previous theories as exclusive, but rather as a combination of intrinsic and extrinsic selective pressures that act for maintenance of sexuality. A short outlook will hopefully stimulate further thinking and future research on plant reproduction.

## ASEXUAL REPRODUCTION IN LAND PLANTS

### *Sexual and asexual reproductive pathways*

In all land plants, the sexual pathway is separated into two multicellular generations: the haplontic gametophyte, which produces male and/or female gametes to conduct fertilization, and the diplontic sporophyte, which develops from the zygote via an embryo stage; the mature sporophyte produces spores via meiosis, and the haploid spores develop again into the gametophyte (Bowman *et al.*, 2016). The plant germline, i.e. the spore mother cells, differentiates late in development on adult plants (Schmid *et al.*, 2015). These developmental features differ fundamentally from animal reproductive development. Among the land plants, only bryophytes are haplontic, i.e. the gametophyte is the predominant phase with pronounced cell differentiation, whereas the other land plants (lycophytes, ferns and seed plants) are diplohaplontic, with a predominant sporophyte and a short-lived gametophyte. In seed plants, the adult plant is the sporophyte, whereas gametophytes are few-celled mini-organisms. The female gametophyte develops within the sporophyte and finally produces the egg cells, whereas the male gametophyte is formed within the pollen grain and acts as a passively mobile carrier of the male gametes. In angiosperms, fertilization is a double process: one male gamete fertilizes the egg cell, and the zygote develops into the embryo; the other male gamete fertilizes the polar nuclei of the central cells, which develop into the endosperm, the nutritious tissue for the embryo within the seed (Fig. 1A). Most important is that a multicellular generation with differentiated cells (complex multicellularity; Bowman *et al.*, 2016), the gametophyte, exists between meiosis and the fertilization process.

Asexual reproduction builds on this generation cycle and modifies it in various ways. Vegetative propagation is not under consideration here, because it starts not from a single cell, but from differentiated tissues and represents a form of growth (Mogie, 1992). Asexual reproduction, involving development from a single-cell stage, can involve different developmental pathways; for instance, a gametophyte can produce an embryo from vegetative tissues (apogamy), a pathway mostly found in ferns and, rarely, in bryophytes (Mogie, 1992). Otherwise, bryophytes use only vegetative propagation as an alternative to sexual reproduction. In ferns, the sporophyte undergoes a modified meiosis to produce unreduced spores. The ploidy level between the sporophyte and the gametophyte is kept at the same diploid level either by a first-division restitution of meiosis, producing diploid spores, or involving a premeiotic endomitosis, resulting in a tetraploid spore mother cell, which then produces diploid spores after reductional meiosis (Grusz, 2016). Premeiotic endomitoses confer a cost on fecundity, because the asexual fern experiences a 50 % reduction of spore production and, consequently, gametophyte and embryo production compared with a sexual one (Mogie, 2013). Asexual ferns are usually female sterile and form neither archegonia nor functional egg cells, and fertilization does not take place. In this way, asexuality could be an adaptation to periodically dry habitats by the complete omission of fertilization, because fertilization is otherwise dependent on water for the active motility of sperm to swim to egg cells (Mogie, 2013). Apogamy is observed mostly in fern lineages in habitats with strong fluctuations of water availability (Grusz, 2016).

In seed plants, a sporophyte can produce embryos from an unfertilized egg cell (parthenogenesis) or from somatic cells of the nucellus (adventitious embryony). Gymnosperms express parthenogenesis only rarely and are otherwise obligate sexual (Mogie, 1992). An aberrant case of paternal apomixis

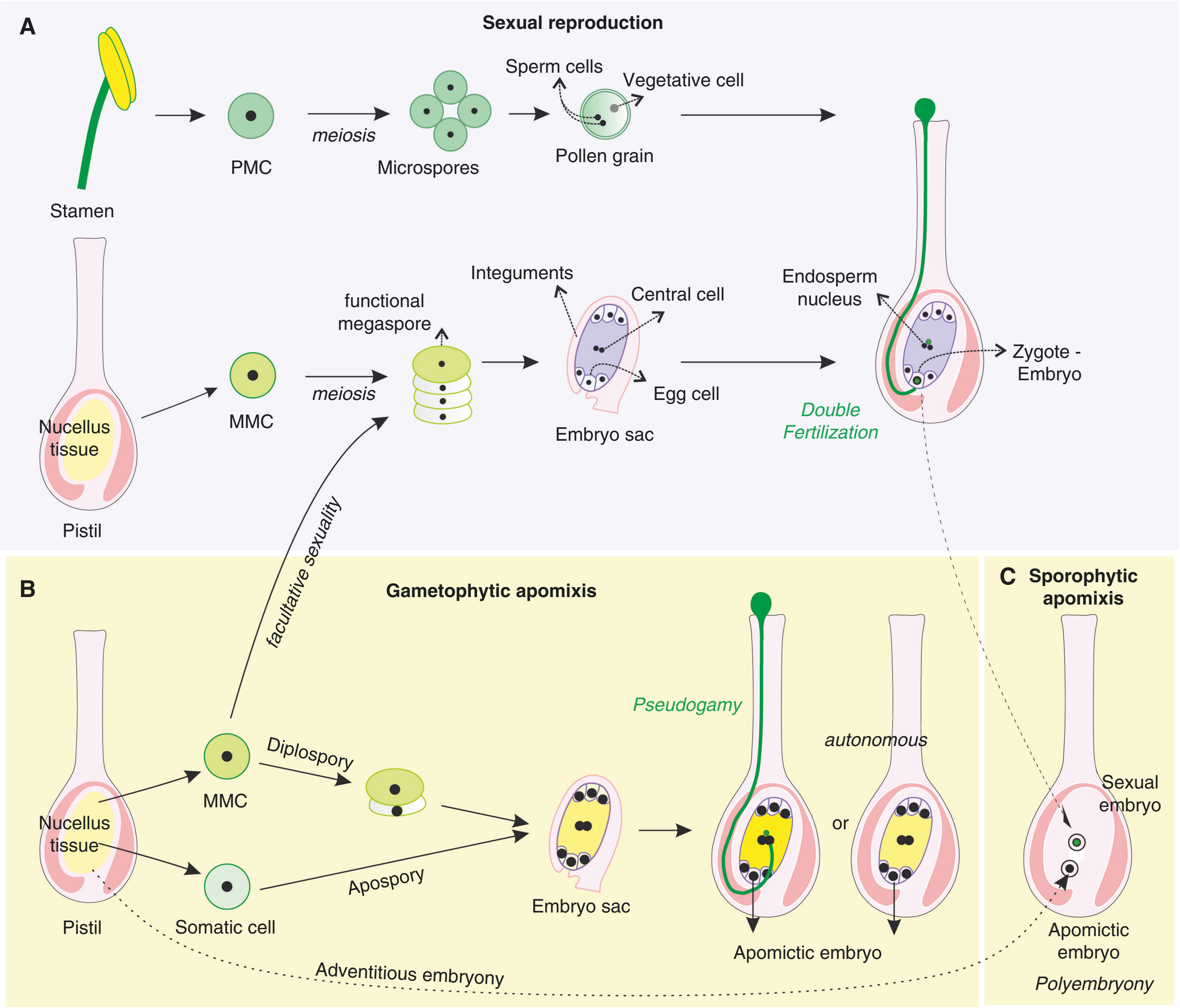

FIG. 1. Developmental pathways of sexual reproduction and apomixis in angiosperms. (A) Sexual reproduction. (B) Gametophytic apomixis, involving the formation of an unreduced female gametophyte (embryo sac) and parthenogenetic development of egg cells; endosperm formation can require fertilization of polar nuclei by sperm nuclei (pseudogamy) or not (autonomous apomixis). (C) Sporophytic apomixis, with embryogenesis from a somatic cell (nucellus or integument) of the sporophyte; often in parallel with sexual development, resulting in sexual + apomictic embryos in one seed (polyembryony). MMC = megaspore mother cell, PMC = pollen mother cell. Redrawn after Mirzaghaderi and Hörandl (2016), copyright 2016 The Authors.

was observed in *Cupressus*, whereby after cross-pollination of two species the pollen grains of the pollen donor underwent androgenetic embryo development within seeds of a 'surrogate' mother of the other species (Pichot *et al.*, 2008). Androgenetic embryo development is also known to be inducible in angiosperms via *in vitro* culture of immature anthers, and this technique is used in breeding programmes to produce highly homozygous double-haploid genotypes (Hale *et al.*, 2022). In gymnosperms, paternal inheritance of cell organelles and the maternal-only production of primary endosperm can enhance natural androgenesis, as observed in *Cupressus*.

In angiosperms, asexual reproduction is called apomixis, defined as reproduction via asexually formed seeds (Asker and Jerling, 1992). Apomixis is widespread taxonomically, and ~2.3 % of angiosperm genera contain apomictic species (Supplementary Data Table S1). Apomixis is derived phylogenetically and occurs in two main non-exclusive forms, gametophytic and sporophytic apomixis (Hojsgaard *et al.*, 2014*b*; Fig. 1B, C). Gametophytic apomixis alters both steps of reproductive development. First, meiosis is either bypassed or altered to be non-reductional (apomeiosis). The bypass of meiosis works via a somatic, unreduced cell of the nucellus, which starts the development of the embryo sac to result in unreduced and genetically unrecombined egg cells (apospory); the meiotic products (megaspores) may be formed in parallel but abort sooner or later. The other pathway is a non-reductional division of megaspore mother cells that results in unreduced spores, from which an unreduced embryo sac is formed (diplospory).

The second step is the bypass of fertilization (parthenogenesis), i.e. the egg cell develops, without the contribution of a male genome, into an embryo that is a clone of the mother plant, with the same ploidy level ($2n$ female and zero male genome contributions, $2n + 0$; Asker and Jerling, 1992; Fig. 1B).

The second major form, sporophytic apomixis, skips only the step of fertilization. Somatic cells of the nucellus or the integuments of the ovule develop directly into an embryo and result in clonal offspring (Fig. 1C). Given that this process often occurs in parallel to sexual development, more than one embryo (a sexual one and one or more apomictic ones) can develop within one seed (polyembryony); the apomictic embryos are called adventitious embryos. The offspring of such a plant might also not be exclusively clonal but might also include recombined genotypes (Asker and Jerling, 1992). Sporophytic apomixis is combined with polyploidy to a lesser extent than gametophytic apomixis and is observed more often in tropical trees. However, gametophytic and sporophytic apomixis are not exclusive but can occur in parallel in the same species (Hojsgaard *et al.*, 2014*b*; Caetano *et al.*, 2018). Some genera with sporophytic apomixis might represent ancient polyploids that have undergone re-diploidization (Carman, 1997).

### *The male function*

These apomictic pathways affect only female development; male meiosis and microsporogenesis are not altered fundamentally and result in meiotically reduced microspores, finally developing into male gametes with reduced chromosome number. In most apomicts, male gametes are still used for fertilization of the polar nuclei for proper endosperm development (pseudogamy; Asker and Jerling, 1992). Only a few plant families, e.g. Asteraceae, have pollen-independent autonomous seed formation (Noyes, 2007). In Asteraceae, tissues other than endosperm in the seed take over the nutritious function, and autonomous apomixis is the rule in this family (Noyes, 2007). In such taxa, male functions can be abandoned and can lead to complete pollen abortion. Nevertheless, even some autonomously apomictic *Pilosella* species do form viable pollen to some degree (Rotreklová and Krahulcová, 2016). Orchids do not produce endosperm in their seeds, hence apomixis is largely pollen independent (Zhang and Gao, 2018). In *Miconia* (Melastomataceae), autonomous apomictic seed formation occurs in parallel with sexual reproduction, thereby maintaining pollen production (Caetano *et al.*, 2018). In apomicts, pollen quality is often variable and usually lower than in sexuals (Hörandl *et al.*, 1997; Ortiz *et al.*, 2013; Schinkel *et al.*, 2017; Conceiçao *et al.*, 2019; Falistocco *et al.*, 2021; Kolarcik *et al.*, 2023), which can usually be referred to meiotic disturbances in hybrid genotypes (Ortiz *et al.*, 2013; Barke *et al.*, 2020), although a certain proportion of functional pollen remains for pseudogamy and/or facultative sexuality.

In many angiosperms, both female and male genome contributions are necessary for proper endosperm formation, with an optimal ratio of 2 maternal to 1 paternal genomes. Deviations from this ratio can result in seed abortion owing to an imbalance of genomic imprinting (Spielman *et al.*, 2003; Vinkenoog *et al.*, 2003). In plants with gametophytic apomixis, deviations from this ratio are observed frequently because of unreduced embryo sac formation, unreduced gamete formation or interploidy crosses (Spielman *et al.*, 2003; Talent and Dickinson, 2007; Aliyu *et al.*, 2010; Schinkel *et al.*, 2017; Kaushal *et al.*, 2018; Kolarcik *et al.*, 2022). However, apomictic plant genera differ in their tolerance of endosperm balance, and some pseudogamous developmental pathways restore the 2m:1p ratio, e.g. by using both sperm nuclei to fertilize the polar nuclei (Talent and Dickinson, 2007; Dobeš *et al.*, 2013; Schinkel *et al.*, 2016). In *Boechera*, disturbed endosperm balance was observed in viable seeds but had an influence on seed size, with larger seeds germinating faster (Paczesniak *et al.*, 2022). Hence, endosperm formation increases phenotypic diversity of seeds among apomictic plants, which selection can act upon (Paczesniak *et al.*, 2022). In most plant families, endosperm formation is essential for embryo survival and germination. Because of the prevalence of pseudogamy, there is positive selection for maintenance of a male function in apomictic plants despite asexual embryo formation (Mogie *et al.*, 2007).

Pseudogamy is often connected to self-fertility, i.e. self-pollen can be used for fertilization of polar nuclei (Hörandl, 2010). On the one hand, this means that a pseudogamous apomict can also reproduce uniparentally, i.e. a single individual can produce fertile seeds without the need for a mating partner or pollinators. On the other hand, via facultative sexuality, sexual seeds can also be formed via selfing (Hörandl, 2010). The proportions of seeds formed by sexual outcrossing vs. sexual selfing in facultative apomicts are unknown.

### *Facultative and partial sexuality*

Taken together, the sexual pathway is not completely lost, because in apomictic plants meiosis is still present, but rather restitutional (diplospory) or bypassed (apospory) during development, and functional reduced male gametes are still formed in most species. This opens opportunities for facultative sexuality, which means that both sexual and apomictic pathways run in parallel in different ovules of the same plant, finally resulting in the formation of both sexual and apomictic seeds by the same parent within the same generation. Proportions of sexual and apomictic seed formation vary considerably between taxa and within taxa, even between years (Aliyu *et al.*, 2010; Sarhanova *et al.*, 2012; Ortiz *et al.*, 2013; Klatt *et al.*, 2016, 2018). Facultative sexuality (or facultative apomixis) maintains genetic variability within offspring and opens vast opportunities for various selective forces, which are discussed below. Overall in angiosperms, sex and apomixis are not black-and-white contrasts, but rather flexible systems with many shades of grey.

The developmental separation of the two major steps (apomeiosis and parthenogenesis) also opens the possibility that only one of the two components is realized. Egg cells can be formed via apomeiosis but then fertilized, resulting in triploid ($2n + n$) embryos and offspring, usually called $B_{III}$ hybrids in the literature (Nogler, 1984). However, meiotically formed egg cells can also develop parthenogenetically (haploid parthenogenesis, $n + 0$ offspring; Nogler, 1984). Such developmental pathways can be named separately as 'partial apomixis' and usually occur at low frequencies (up to ~2 %) of offspring (Bicknell *et al.*, 2003; Bicknell and Koltunow, 2004; Aliyu *et al.*, 2010; Schinkel *et al.*, 2017). Partial apomixis is not stable over generations, because selection would act against continued polyploidization in $B_{III}$ lineages owing to cellular constraints, whereas haploid parthenogenesis is constrained by the expression of negative recessive mutations that were previously buffered in the diploid mother plant (Van Dijk and Vijverberg, 2005). Although partial apomixis pathways occur at low frequencies, they are evolutionarily relevant. The embryos differ in ploidy level from the mother plant and also from their sexually and apomictically formed siblings, which might open opportunities for polyploidization via a female triploid bridge with unreduced, apomeiotically formed female gametes (Schinkel *et al.*, 2017) or evolution of apomixis via mutated haploid parthenogenetic plants (Bicknell *et al.*, 2023). Hence, partial apomixis can result in new asexual lineages and contribute to clonal diversity.

## THE COSTS AND BENEFITS OF MEIOSIS

### *Meiosis and recombination*

Meiosis is the shared key process of sexuality in eukaryotes (Brandeis, 2018), and the meiosis gene machinery is present in all eukaryotes (Hofstatter and Lahr, 2019; Hofstatter *et al.*, 2020). Meiosis involves the pairing of homologous chromosomes, eventually resulting in crossing over and meiotic recombination. The fusion of two gametes from different parents results in gametic recombination. The benefits of recombination were traditionally seen as an advantage of meiosis by producing genetic variation in the offspring, upon which selection can act more efficiently. However, it was recognized in the 1980s that recombination is not necessarily a selective advantage, but effects of recombination depend on the selection scenario and the strength of selection (Brooks, 1988). Recombination can break up well-adapted genes and might even be selected against, and its advantages alone are not strong enough to explain obligate sexuality under assumptions of variable selection regimes (Otto, 2009; Brandeis, 2018). Moreover, meiosis is a time-consuming, risky process that is prone to errors. The model of potential advantages of individual genetic variation is not easily applicable to plants, because most asexual land plants are at the same time polyploids (Asker and Jerling, 1992; Carman, 1997; Hojsgaard *et al.*, 2014*b*; Grusz, 2016). Polyploidy results in higher intragenomic diversity, allelic diversity and epigenetic flexibility, which allows asexual plants to respond efficiently to environmental selective pressures and stresses despite reduced individual genetic diversity (Comai, 2005; Fox *et al.*, 2020; Van de Peer *et al.*, 2021). For instance, adaptation to extreme environmental conditions can be achieved in asexual polyploids by phenotypic plasticity and by flexibility in epigenetic control mechanisms of adaptive traits (Verhoeven and Preite, 2014; Syngelaki *et al.*, 2020, 2021). In many plant genera, the apomictic lineages have much larger distribution areas than their sexual congeneric relatives (geographical parthenogenesis), which can mostly be referred to advantages of uniparental reproduction, but also to ecological niche shifts (Hörandl, 2023). Niche shifts might be conducted by asexual polyploids better than by sexual populations e.g. (Kirchheimer *et al.*, 2016; Karunarathne *et al.*, 2018) they might at least have equal niche dynamics to sexuals, as also shown in invasive plants (Dellinger *et al.*, 2016). In this way, polyploid apomicts contradict the assumptions of the classical ‘tangled bank’ hypothesis that sexual reproduction, by providing genotypic variation, would be advantageous in spatially heterogeneous environments (Bell, 1982). In angiosperms, it appears that exogenous selection by the environment acts more efficiently on cytotypes than on individual genotypes. This altered genomic background might also explain why the so-called Red Queen model [i.e. a better response of sexual organisms to biotic stressors (parasites, pathogens and predators) because of higher genotypic variability compared with asexuals] could not be confirmed for plants (Hartmann *et al.*, 2017). Polyploid genomes of asexual plants can also improve the response to biotic stressors (Van de Peer *et al.*, 2021).

Furthermore, flowering plants often exhibit facultative sexuality. It has been shown both theoretically and empirically that a little bit of sex (5–10 %) is good enough to respond to selective forces under environmental variability (D’Souza and Michiels, 2010), and this prediction also holds for polyploid plants. In theory, facultative sexuality would combine the benefits of both reproductive systems: maintenance of well-adapted genotypes as clones via apomixis and occasional recombination and variation to respond to environmental variability (D’Souza and Michiels, 2010). This prediction also holds for polyploid apomicts (Hörandl, 2023). The question arises, why are these flexible systems of facultative apomixis not predominant in all plants? The main conclusion is that advantages of genetic recombination and individual genetic variability are not the major selective forces for maintenance of sex, but rather a positive side effect of a process that has evolved because of the much stronger and continuous intrinsic selective pressure of DNA restoration from generation to generation (Hörandl, 2009).

### *DNA restoration theories*

The genome is continuously subject to physical damage and mutations, with most of them being disadvantageous (Friedberg *et al.*, 2006). The transgenerational transmission of an integer genome is an essential and continuous selective pressure. For this purpose, meiosis might serve as a 2-fold DNA restoration tool (here used as an umbrella term; Hörandl, 2009), namely: (1) as a tool for homologous recombinational repair of physical DNA damage during prophase I of meiosis; and (2) as a mechanism for the selective elimination of negative mutations via recombination and ploidy cycling. Both processes have immediate positive organismal effects (Kondrashov, 1993). Both processes act together in germline cells to guarantee inheritance of integer nuclear DNA to the next generation and might balance the above-mentioned ‘costs of meiosis’.

### *Meiosis evolved as a DNA repair tool*

DNA is continuously damaged, mostly by oxygen radicals produced endogenously by the major metabolic processes in eukaryotes (i.e. aerobic respiration via mitochondria) and in plants additionally by photosynthesis. Among the many known DNA cellular repair mechanisms, non-homologous repair is less accurate and can result in mutations, whereas homologous recombinational repair is the most accurate and least mutagenic process (Ranjha *et al.*, 2018). During meiosis, double-strand breaks (DSBs) of DNA occur, and homologous recombinational repair of these breaks takes place by using the homologous chromosome preferentially, instead of sister chromatids (Ranjha *et al.*, 2018). This process requires a homologous DNA strand with a different history of damage, which is achieved during meiosis via pairing of homologous chromosomes derived from different parents. At the evolutionary origin of eukaryotes, this requirement for homologous DNA might have been a driver for mating and combining two parental genomes (Hörandl and Speijer, 2018).

In extant plants, homologous pairing starts with centromere and telomere tethering at the nuclear envelope. The installation of the synaptenomal complex establishes the axis of chromosome pairs, and during this process DSBs are formed as sites for initiation of recombination (Sepsi and Schwarzacher, 2020). However, most DSBs at meiosis result in non-crossovers (i.e. in non-reciprocal recombination) in which a small part of the intact homologous chromosome is copied to the broken chromosome (Mercier *et al.*, 2015). However, only crossovers (reciprocal recombination) result in substantial recombination. The meiosis machinery is not at all adapted to maximize genetic recombination, because most DSBs during the onset of meiosis I do not result in crossing over and recombination of flanking regions (Bernstein *et al.*, 1988, Bernstein and Bernstein, 1991, 2013). In plants, the number of DSBs exceeds the number of crossovers 10- to 50-fold (Mercier *et al.*, 2015), which means that selection for recombination cannot explain the initiation and primary function of meiosis. Instead, repair of previous DNA lesions caused by reactive oxygen species is likely to be the primary function of meiosis initiation during prophase I (Hörandl and Hadacek, 2013). In this model, DSBs are not 'programmed' but are induced biochemically by reactive oxygen species-induced DNA lesions that arose during overall stress conditions in the organism (Hörandl and Hadacek, 2013). According to this model, even minor oxidative lesions of DNA (e.g. a free electron) could activate a key enzyme of meiosis in germline cells, Spo11, to scavenge free DNA radicals with its antioxidative tyrosine end; this redox reaction leads consequently to a DSB at the sugar–phosphate backbone of DNA (Hörandl and Hadacek, 2013). Afterwards, the homologous recombinational meiosis machinery will repair this break, as described by Mercier *et al.* (2015), but result in only a few cases in crossovers and genetic recombination. At least one crossover per bivalent is necessary for proper segregation of chromosomes, but it rarely exceeds three per bivalent (Mercier *et al.*, 2015). The much lower frequency of crossovers in comparison to initial DSBs implies that a mechanistic selective pressure acts to retain a minimum number of crossovers rather than to increase recombination rates. For animals, comparison of the evolution of autosomes and sex chromosomes supports the theory that the major function of meiosis is to retain genomic integrity (Brandeis, 2018). Genetic recombination is a by-product of meiosis, which might be under positive or negative selection later on (Hörandl, 2009).

The DNA repair function was probably established during the origin of eukaryotes, when aerobic respiration via mitochondria started and strongly increased endogenous oxidative stress (Speijer *et al.*, 2015; Speijer, 2016; Hörandl and Speijer, 2018). Plants also have plastids and conduct photosynthesis, which is a major source of reactive oxygen species. Evidence has emerged in recent decades that sex is ancestral in eukaryotes. First, many protists have sexual cycles, which can be stimulated by DNA damage, and use meiosis as a DNA repair tool (Ramesh *et al.*, 2005; Schurko and Logsdon, 2008; Bernstein *et al.*, 2018; Da Silva and Machado, 2022). Second, the 'meiosis toolkit' and key genes for plasmogamy were already present in the common ancestor of eukaryotes and are found in all eukaryotic lineages, even in taxa that were thought for a long time to be asexual (Schurko and Logsdon, 2008; Speijer *et al.*, 2015; Speijer, 2016; Hofstatter and Lahr, 2019; Hofstatter *et al.*, 2020). Third, most developmental pathways of asexual reproduction in plants and animals do keep homologue chromosome pairing at prophase I of meiosis as an indispensable DNA repair tool (Grusz, 2016; Mirzaghaderi and Hörandl, 2016). Fourth, plants and animals with facultative or cyclical asexuality show higher frequencies of sexuality in stressful environmental conditions (Ram and Hadany, 2016; Niccolò *et al.*, 2023), supporting the hypothesis that oxidative stress induces meiosis. Comprehensive experimental and molecular work on facultative apomictic plants has confirmed that environmental stressors (light, heat, drought, salt and osmotic stress) triggers sexual reproduction in plants via epigenetic control mechanisms of the metabolic stage and the stress response, and interacting with genetic control of reproductive genes (reviewed by Niccolò *et al.*, 2023).

### *Elimination of mutation via meiosis functions*

Mutation, as a change in the sequence of DNA bases, is a result of incorrect DNA repair, mostly occurring at end-joining, which does not require a homologous template (Ranjha *et al.*, 2018). Mutations, most of which have negative or deleterious effects, are a ubiquitous threat for genomic integrity and inheritance of functional genomes. The accumulation of deleterious mutations over generations has been recognized as a potential risk for extinction of asexual lineages (Muller's ratchet) (Muller, 1964; Kondrashov, 1988). Mutations cannot be repaired actively; they can only be eliminated by selection against their negative effects. Recombination via sex increases the efficacy of selection against negative mutations and is regarded as the major mechanism counteracting genomic decay in eukaryotes (Colnaghi *et al.*, 2020). The rationale is that recombination can result in genotypes with a lower mutational load and exposes genotypes with a high mutational load to purifying selection. In the long term, asexual reproduction without any recombination would result in genomic decay owing to the accumulation of deleterious mutations, specifically in small populations, finally leading to extinction of the asexual lineage.

Traditional evolutionary theory regarded these aspects as a major advantage to meiotic sex in eukaryotes, but empirical genomic data on the accumulation of mutations in asexual eukaryotes are scarce and equivocal (Glemin *et al.*, 2019; Hörandl *et al.*, 2020; Jaron *et al.*, 2020). Several asexual animals and plants do not show genome-wide accumulation of mutations (Flot *et al.*, 2013; Pellino *et al.*, 2013; Brandt *et al.*, 2017; Kočí *et al.*, 2020) or show it only in less conserved genes (Lovell *et al.*, 2017). However, the datasets are derived from a few model systems; they are based, in part, on transcriptomes only and might also be biased methodically. In the last few years, evidence has emerged that the classical methods of estimating selection on coding DNA by calculating ratios of non-synonymous (dN) to synonymous mutations (dS) need to be interpreted with caution (Del Amparo *et al.*, 2021).

For diploid sexual plants, the cycling of ploidy levels between multicellular gametophytes (*n*) and sporophytes (2*n*) is probably a major selective force against the accumulation of deleterious mutations, acting in addition to recombination (Fig. 2A). During the diplontic stage (sporophyte), recessive mutations on one allele will be buffered by the unmutated allele and therefore 'masked' (i.e. not expressed, hence not exposed to selection). After reductional meiosis, these mutations will be exposed to purifying selection in the haplontic stage, i.e. in the gametophytes. Haploid purifying selection might be strong in haplontic and haplodiplontic organisms, but weakened in diplontic or diplohaplontic organisms; asexuals without ploidy cycling miss this selection phase (Otto and Gerstein, 2008; Gerstein and Otto, 2009). Furthermore, beneficial mutations would also be stronger under positive selection in the haplontic phase (Otto and Gerstein, 2008; Gerstein and Otto, 2009). Following this rationale, haplontic algae and haplodiplontic bryophytes would experience regular purifying selection of negative mutations and rapid diversifying selection on beneficial mutations in their main life phase, the gametophyte. In the diplohaplontic ferns, the gametophyte is still a free-living, photosynthetic multicellular organism with full gene expression (Wyder *et al.*, 2020), favouring haploid selection against negative mutations. Apogamy emerged in ferns several times in the phylogeny, is thought to occur in evolutionarily young lineages (≤8 Myr old) and is strongly associated with polyploidy (Liu *et al.*, 2012). The lack of a ploidy cycle in apogamous ferns might result in accumulation of recessive mutations, which

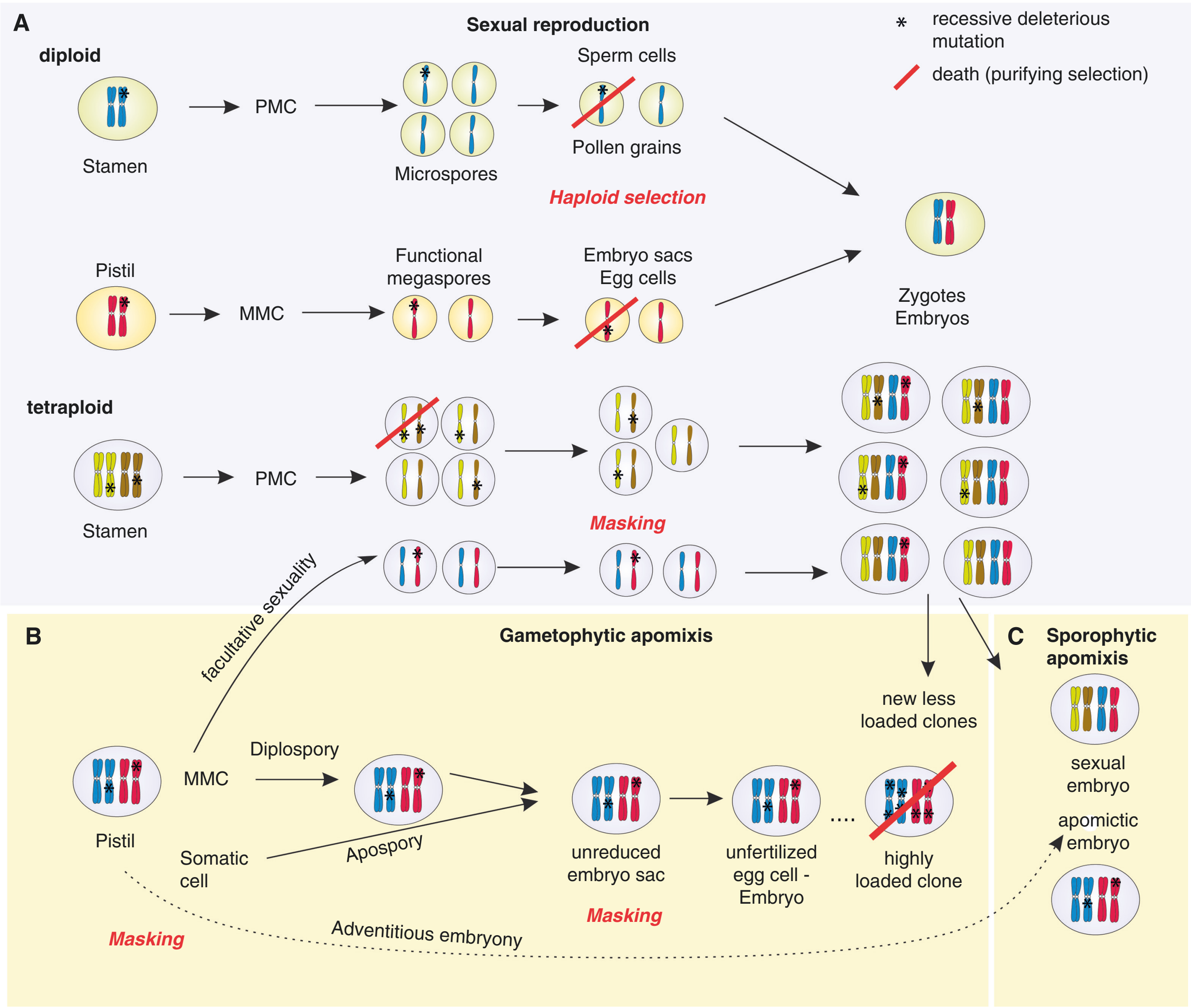

FIG. 2. Cytological processes and hypothetical mutational dynamics in sexual and apomictic angiosperms. (A) Sexual reproduction of a diploid, with haploid selection against recessive deleterious mutations during gametophyte phase; in a sexual tetraploid, recessive mutations (one allele) would be buffered by the unmutated allele, hence masked from selection, but nevertheless, recombination can result in unmutated genotypes and keep the mutational load low. (B) In gametophytic apomixis, mutations are also masked, but accumulate over generations (…), but facultative sexuality could reduce the mutational load and generate a clone with less loaded genotypes. (C) Sporophytic apomixis runs in parallel with sexual reproduction and is similar to B. MMC = megaspore mother cell, PMC = pollen mother cell.

could be buffered for some time by polyploidy. A transcriptome study on the obligate asexual fern *Vittaria appalachiana*, which exists only as a gametophyte, suggests decreased efficacy of purifying selection, particularly in genes related to the cell cycle, and altered patterns of transposable elements; two rounds of polyploidization in the evolutionary history might have allowed persistence of the lineage (Pelosi *et al.*, 2023).

In seed plants, the female gametophytes are reduced to few-celled, organ-like mini-organisms that are no longer metabolically independent and develop within the sporophyte, whereas the few-celled male gametophyte develops within in the pollen grain. Nevertheless, haploid selection is expected to be efficient because a high proportion of the genome is still expressed in gametophytes, and gene expression patterns of gametophytes and sporophytes show large overlaps (Immler and Otto, 2018; Beaudry *et al.*, 2020; Somers and Nelms, 2023). In angiosperms with gametophytic apomixis, the female gametophyte is unreduced and the effect of haploid selection weakened (Fig. 2B). Negative recessive mutations would be masked by the unmutated allele and would keep the respective gene functional, hence the mutation would not expose it to purifying selection (Hodač *et al.*, 2019). The effects of selection on mutations in polyploids depends on their level of dominance (Gerstein and Otto, 2009). Pollen is meiotically reduced, but given that most apomicts are polyploid, they would produce mostly diploid pollen (or higher with ploidy levels), in which the masking effect on recessive mutations would still be present (Beaudry *et al.*, 2020). The mutational dynamics in apomictic polyploids is seen in allelic sequence divergence, which occurs even in young (80 000 years old) lineages (Pellino *et al.*, 2013).

For apomictic angiosperms, facultative sexuality is probably the main evolutionary strategy to avoid long-term genomic decay. Proportions of sexually vs. asexually formed seeds vary not only within a generation, but also between different years in perennial plants, as has been shown, e.g. in the alpine species *Ranunculus kuepferi* (Klatt *et al.*, 2018). A study on polyploid offspring of plants of the facultative apomictic *Ranunculus auricomus* complex revealed that a little bit of sex (a mean of ~6 % recombined offspring in three progenies) is sufficient to avoid accumulation of mutations over generations (Hodač *et al.*, 2019). The efficiency of a low frequency of sex might be increased strongly by selection on gametophytes. Empirical data on the developmental stages suggest that strong purifying selection is acting in the haplontic female gametophyte by reducing the proportions of sexually formed seeds compared with apomictic seeds from the same mother plant (Hodač *et al.*, 2019). However, purifying selection might also act in the diplontic phase of the life cycle, depending on allele dosages and dominance levels of mutated alleles (Hodač *et al.*, 2019). Similar results were obtained for sporophytic apomixis in Citrinae, for which whole-genome analyses revealed elevated levels of deleterious mutations in the heterozygous state, hence these mutations are masked; also in this case, facultative sexuality can counteract accumulation of mutations and genomic decay in wild populations (Wang *et al.*, 2022; Fig. 2C). Facultative apomixis is also common in other families with sporophytic apomixis (Mendes-Rodrigues *et al.*, 2005; Mendes-Rodrigues and Oliveira, 2012; Viana *et al.*, 2021).

After selfing, previously accumulated heterozygous recessive mutations of the sporophyte will become homozygous in the offspring, hence also exposing negative mutations more efficiently to purifying selection (Glemin *et al.*, 2019). Genome studies on selfing organisms do not show relaxed selection on the accumulation of mutations (Glemin *et al.*, 2019). This potential purging mechanism has not yet been studied in apomictic plants but deserves attention because most apomictic pseudogamous plants are self-fertile (Hörandl, 2010). Self-fertility does not mean that self-pollination takes place only within the same plant individual, but it also extends to clones; occasional ‘cross-fertilization’ between individuals sharing the same genotype (clone-mates) will also result in genetically selfed progeny with increased homozygosity. Sexual selfing in diploid plant lineages will allow for selection on homozygous mutations in the sporophytes and thus reduce the relative efficacy of haploid selection in the gametophyte (Beaudry *et al.*, 2020). In facultative apomicts, however, polyploidy of a clonal lineage could buffer the effects of accumulation of a recessive mutational load for several generations (Gerstein and Otto, 2009). However, after a rare sexual selfing event, which makes many mutations homozygous and fully expressed to selection in some fraction of the offspring, this would cause the death of a highly loaded genotype via truncating selection (Hodač *et al.*, 2019). Such deaths would eliminate a high number of negative mutations from that lineage in one step (Kondrashov, 1988).

In this way, selection mechanisms via ploidy cycling are probably more efficient in diplohaplontic seed plants than in diplontic animals, in which a prolonged multicellular haplontic life phase is missing and in which selfing is less common, even in hermaphrodites. Theoretically, some forms of automixis in asexual (parthenogenetic) animals would result in an increase of homozygosity and could have similar purging effects (Engelstädter, 2017). Obligate asexual lineages in plants and animals without any meiosis appear to be atypical and exceptional cases for their respective kingdom. For instance, bdelloid rotifers have specific adaptations to extreme environmental stress (ultraviolet radiation and desiccation) to avoid oxidative damage, and their meiosis toolkit has changed its function to repair DNA DSBs (Hecox-Lea and Mark Welch, 2018). The functional asexual plant *Oenothera biennis* exhibits a rare form of meiosis (translocation heterozygosity without homologue pairing and segregation) and appears to exhibit signs of accumulation of mutations in the genome (Hollister *et al.*, 2015). However, this species has kept the meiosis machinery and has rare recombination events (Hofstatter and Lahr, 2019). Further candidates for obligate apomixis (apospory combined with autonomous apomixis) can be found in the genus *Alchemilla* (Rosaceae). Here, extreme high ploidy levels (≤16×) might allow for a buffering of the mutational load, at least for the lineages observed today. The progenitors and age of these apomictic lineages are not yet known (Gehrke *et al.*, 2016). A major difficulty in estimating the long-term evolutionary success of such asexual lineages is that plant fossils do not provide information on the mode of reproduction; therefore, age estimates for the time of shifts to asexuality and for presence of asexuality over time within a lineage cannot be made.

### *Organelle–nuclear genome compatibility; an overlooked factor?*

For many decades, the effects of modes of reproduction were considered only for the nuclear genome. However, the organelles and organelle–nuclear interaction might play a role in the mode of reproduction. During eukaryogenesis, an archaeal host incorporated an aerobic alpha-proteobacterium (or closely related lineages) that became the mitochondrion (Timmis *et al.*, 2004); in a second endosymbiosis, a protist incorporated a free-living cyanobacterial plastid progenitor with oxygenic photosynthesis (Timmis *et al.*, 2004). Both endosymbioses increased intracellular oxidative stress and oxidative DNA damage, which might have triggered evolution of the nucleus and chromosome structure in early eukaryotes and of meiotic sex as a DNA restoration tool (Speijer, 2016; Hörandl and Speijer, 2018). Massive endosymbiotic gene transfer from these organelle genomes to the nucleus resulted in a chimeric nature of eukaryotes, with a recombinant and biparentally inherited nuclear genome and with largely non-recombinant, usually uniparentally (maternally) inherited and small organelle genomes (Ku *et al.*, 2015). After uptake of endosymbionts, their prokaryotic genome (and their independence) was reduced through endosymbiotic gene transfer by ≥90 % (Martin and Herrmann, 1998; Timmis *et al.*, 2004; Archibald, 2015; Qiu *et al.*, 2017). Uniparental inheritance of organelles probably evolved very early in eukaryotes (Speijer, 2016) and has direct consequences for reproductive biology of eukaryotes. The co-dependency of the cell and the organelle necessitates a tight coordination between the nucleus and the organelle.

For nuclear–organelle interactions, it is unclear whether sex provides a general advantage or disadvantage. Havird *et al.* (2015) proposed a novel hypothesis of nuclear–mitochondrial co-evolution as a driver for the evolution of sex: high mutation rates and accumulation of mutations in mitochondrial (mt) genomes require recombination of the nuclear-encoded genes for mitochondrial functions as a compensatory mechanism for mt decay. However, sex does not necessarily have only positive effects on cyto-nuclear complexes. Outcrossing can increase the conflict between genomes, because the associations of alleles in the nucleus are only temporary, and differences in transmission between nuclear and cytoplasmic genes can give rise to conflict, thereby reducing fitness of sexuals in comparison to asexuals (Partridge and Hurst, 1998).

Plant mt genomes are larger and more complex than in animals and encode ~20–40 genes (Møller *et al.*, 2021). Plant mt genomes have a low divergence, but higher diversity owing to many repeats and high recombinational activity. The mtDNA of plants is partitioned into many small chromosomes that can undergo homologous recombination, which increases the efficiency of DNA repair (Møller *et al.*, 2021). These processes could explain the avoidance of ageing effects and longevity of some plants, especially of trees, but also the relative high resistance of plants to abiotic stress. Plastome evolution is even more complex, because plastids have spread horizontally multiple times (i.e. across species boundaries) through secondary endosymbioses (recently reviewed by, e.g. Sibbald and Archibald, 2020). Plastome mutation rates are usually lower than nuclear ones, but a few studies support plastid–nuclear compensatory co-evolution analogous to mt evolution (Hill, 2020).

In sexual plants, coadaptation of both nuclear and organellar genomes might act as a crossing barrier, hence it could be a strong factor in speciation (Postel and Touzet, 2020). Theoretically, apomictic reproduction would keep coadapted nuclear–organelle associations together in clonal lineages, potentially resulting in higher fitness. Such an association has been observed in selfing plants (Postel and Touzet, 2020) but has not yet been studied for apomictic plants. However, hybridization and/or polyploidization of previously isolated lineages can result in cyto-nuclear conflict, as has been observed in some polyploid sexual plants (Postel and Touzet, 2020). This could influence selection processes in apomictic lineages of allopolyploid origin, but no empirical studies are yet available.

Following predictions by Havird *et al.* (2015), eukaryotes with low mt mutation rates should exhibit lower propensities for sexual reproduction, because with a low mt mutation rate, selection for sex as a compensatory mechanism for the nuclear genome should be relaxed. The evolution of the mt genome in apomictic plants is basically unknown, but some plastome data are available. Low mutation rates of organellar genomes would also facilitate hybridization between lineages via facultative sex, because a coadapted cyto-nuclear complex would be shared between them. This hypothesis is in line with the empirical observation of high gene flow between facultative apomictic lineages and low phylogenetic divergence of plastome data within the polyploid apomictic *R. auricomus* complex (Karbstein *et al.*, 2022). Low divergence of plastomes was also apparent within the sections of the genus *Crataegus*, whereas nuclear gene phylogenies were better resolved and evidenced hybridization and allopolyploidy (Liston *et al.*, 2021). However, in *Cotoneaster* the plastomes resulted in fully resolved trees with incongruence to nuclear trees owing to hybridization and polyploidization (Meng *et al.*, 2021). Low substitution rates were found in plastomes of *Cotoneaster* (Yang *et al.*, 2022) and in apomictic microspecies of *Taraxacum* (Salih *et al.*, 2017). Much more work is needed to understand cyto-nuclear interactions in apomictic plants.

## THE COSTS AND BENEFITS OF SEPARATE SEXES IN PLANTS

Classical theories derived from the animal kingdom postulate a high cost of sex because of male individuals that would not produce offspring themselves. A female asexual individual would produce twice as many offspring as a sexual female. However, mating strategies are highly diverse across eukaryotes (Brandeis, 2018). For plants, this 'cost of males' is not well applicable, because most species are hermaphrodites, with sporophytes bearing stamens and carpels either within the same flower or on different flowers of the same individual (monoecy); various other combinations of sexes exist (Richards, 1997). Combined sexes are probably a major advantage for reproductive assurance in sessile organisms, because no active movement and mate selection can be performed (Hörandl and Hadacek, 2020). Dioecy with male and female plants occurs in only 5–6 % of angiosperm species and is selected positively in long-lived, often wind-pollinated trees to avoid continued self-fertilization (geitonogamy; Renner, 2014). If asexuality were to be a selective advantage for species with different sexes, as postulated by the 'cost of male' theory, one would expect elevated frequencies of apomixis in dioecious plants. From the 986 genera with dioecious species (Renner, 2014), only 42 have at the same time apomictic species (4.3 %); only 13 genera have apomixis and sex chromosomes [Supplementary Data Table S1; data for sex chromosomes after Baránková *et al.* (2020); for apomixis after Hojsgaard *et al.* (2014*b*), Souza-Perez and Speroni (2017), Brožova *et al.* (2019), Mandakova *et al.* (2020), Pegoraro *et al.* (2020), Mandakova *et al.* (2021), Xiao *et al.* (2021) and Reposi *et al.* (2023); reviewed by Hojsgaard and Pullaiah (2023)]. The data would not suggest a tendency for dioecy to enhance shifts to apomixis. The associations would be much lower at the species level than the genus level, because both apomixis and dioecy are rarely exclusive traits within a genus, but data on co-occurrences at the species level are far too incomplete for the estimation of correlations.

Hence, the costs and benefits of a male function in plants have to be seen in a completely different way from those in animals. In a hermaphroditic plant, pollen production requires much less resources than seed production, hence outcrossers have high pollen-to-ovule ratios (Richards, 1997). Pollen production is 'cheap' and therefore also not a major cost to apomictic plants. The great majority of apomictic plants are functionally hermaphroditic and pseudogamous (see above), and usually at the same time self-fertile (Hörandl, 2010; Cosendai *et al.*, 2013). The breakdown of the self-compatibility system in apomicts is probably attributable to polyploidy and does not have a direct functional connection to apomixis. However, selection will favour self-compatible genotypes in apomicts, because otherwise pollination within clonal lineages between clone-mates (i.e. between neighbouring individuals of a clone) would fail (Hörandl, 2010). Self-fertility also means that apomictic plants, as sessile organisms, can benefit from self-pollination for reproductive assurance. Interestingly, apomictic plants do not show morphological adaptations to selfing (i.e. a selfing syndrome with reduced flower size), which is well known from sexual selfing plants (Glemin *et al.*, 2019). Pollen-to-ovule ratios are difficult to assess, because pollen fertility in apomicts is often lower than that in sexuals owing to varying proportions of malformed pollen (e.g. Hörandl *et al.*, 1997; Schinkel *et al.*, 2017; Kaushal *et al.*, 2018). In apomictic plants, self-pollination is probably not obligate, but little is known about this aspect. However, these aspects favour maintenance of a male function in apomicts.

Maintenance of the male function in apomictic plants will also be selected for maintenance of facultative sexuality, which counteracts accumulation of mutations (see above). Furthermore, reduced pollen can transfer infectious apomixis to sexual plants, especially when ploidy levels of the pollen donor and the recipient are the same, such as in *Boechera* (Mau *et al.*, 2021).

Overall, maintenance of male functions has benefits for plants, probably balancing its costs. The main cost of pollination in plants is the production of flowers, which also applies to pseudogamous plants and is a cost of the functionally hermaphroditic sporophyte (hence no 'cost of males'). Flower size is obviously not reduced in apomictic plants (Whitton *et al.*, 2008), other than in selfing plants (Cutter, 2019). Some reductions of floral displays have been observed in apomictic *Ranunculus* species (Huber, 1988; Karbstein *et al.*, 2021; Melzheimer and Hörandl, 2022) and in functionally asexual *Oenothera* (Johnson *et al.*, 2010) but are not documented from other genera. The main reasons for maintaining floral displays could be the prevalence of pseudogamy, for which insect visits are still advantageous for pollen dispersal. Even with self-fertility, insects would distribute pollen by moving around within a flower or visiting different flowers of the same individual (geitonogamy). In sexual plants, large floral displays can be disadvantageous because of the risk of self-pollination via geitonogamy, which would have the disadvantage of sexual self-fertilization and loss of heterozygosity in the offspring (Harder and Barrett, 1995). Pseudogamous self-pollination does not confer this disadvantage, because egg cells are not being fertilized, but it would have the full advantage of uniparental seed formation. Little research has been done on the frequencies and effects of pseudogamous self-pollination in plants.

## CONSTRAINTS OF NATURAL SHIFTS FROM SEX TO APOMIXIS

Once the sexual cycle of meiosis and fertilization has been established, it is difficult to get rid of it, because alternating only one step cannot establish a permanent reproductive system; apomeiosis with fertilization would result in continued increase of ploidy levels, which is limited by cellular constraints, whereas meiosis combined with parthenogenesis results a haploid stage from which a further reduction is not possible (Van Dijk and Vijverberg, 2005). Selection acts against partial apomixis as a transition stage. Only the concomitant alternation of both developmental steps is expected to be stable, which might be a major reason for the rarity of apomixis at the species level. In angiosperms, the shift to apomixis is relatively well studied. Apomixis represents a modification of the sexual pathway (Fig. 1) and is based on the same genes that are regulated differentially (Leon-Martinez and Vielle-Calzada, 2019; Schmidt, 2020; Niccolò *et al.*, 2023). The genetic factors for gametophytic apomixis are heritable and include at least two unlinked genetic control factors, one for apomeiosis and one for parthenogenesis (Ozias-Akins and van Dijk, 2007).

Natural apomixis arises mostly in interspecific hybrids, which was first proposed by Ernst (2018) and has been confirmed in numerous case studies [reviewed by Hojsgaard and Hörandl (2019); further studies by Liston *et al.* (2021); Mandakova *et al.* (2021); Karbstein *et al.* (2022); Lewin *et al.* (2022); Bradican *et al.* (2023) and Hajrudinovic-Bogunic *et al.* (2023)]. Evidence is accumulating that hybridization at the diploid level is the first step for triggering apomeiosis, as shown experimentally in *Ranunculus* (Hojsgaard *et al.*, 2014*a*, Barke *et al.*, 2018) and observed in many natural apomicts in Boechereae in Brassicaceae (Mandakova *et al.*, 2021). Occasional, rare apomictic seed formation was also found, for instance in diploid progenitors of autotetraploid, apomictic *R. kuepferi* (Schinkel *et al.*, 2016) and in *Paspalum rufum* (Soliman *et al.*, 2021). Notably, apospory and diplospory appear occasionally in otherwise sexual populations, but without the connection to parthenogenesis (Noyes, 2007; Ortiz *et al.*, 2013). Both genetic and epigenetic control mechanisms appear to be involved in the bypass of meiosis (Leon-Martinez and Vielle-Calzada, 2019; Schmidt, 2020). Gene expression profiles are specific for the different developmental steps from pre-meiosis to embryo sac formation and differ between sexual and apomictic plants (Sharbel *et al.*, 2009, 2010; Galla *et al.*, 2017; Garbus *et al.*, 2017; Rodrigo *et al.*, 2017; Pellino *et al.*, 2020; Soliman *et al.*, 2021). However, only a small fraction of interspecific hybrids in plants shift to apomixis, considering that ~25 % of species in angiosperms do hybridize but either remain as a sterile F1 or evolve further by sexual reproduction (Arnold, 1997; Mallet, 2007). It appears that either additional cytogenetic changes (Kantama *et al.*, 2007) or a 'lucky combination' of mutations in newly formed hybrids (Paetzold *et al.*, 2022) are causal factors for stable shifts to apomeiosis at the diploid level. Environmental effects have been observed on expressivity of apomeiosis, but not on parthenogenesis (Klatt *et al.*, 2016; Soliman *et al.*, 2021; Ulum *et al.*, 2021). Irregularities of meiosis in homoploid hybrids might play a role in expression of apomixis but appear at frequencies too low to provide the sole explanation of a selective pressure for the shift to apomeiosis in hybrids (Barke *et al.*, 2020; Falistocco *et al.*, 2021). However, considering the tight control that is necessary for exact homologous pairing and recombination, the danger of mispairing and ectopic recombination will increase in polyploids because of their multiple chromosome sets (Sepsi and Schwarzacher, 2020). In allopolyploids, recombination between homeologous chromosomes (those from different parents, i.e. from different subgenomes) can cause cytological problems, and genetic mechanisms drive preferential recombination between homologous chromosomes (those coming from the same parent) (Bomblies, 2023). In autopolyploids, chromosomes are derived from the same genome and lack preferential pairing partners; here, the problem is often formation of multivalents during prophase I, resulting in irregular segregation in anaphase I and II (Bomblies, 2023). Hence, apomixis could be favoured selectively by avoiding potential negative effects of meiosis irregularities in newly formed polyploids.

Apomixis in autopolyploids can be induced environmentally or genetically. Cold treatments triggered apomictic seed formation in otherwise sexual diploid plants of the alpine species *R. kuepferi* (Klatt *et al.*, 2018). In natural populations of the species occurring in areas with colder climates, unreduced egg cell formation allowed for polyploidization via a female triploid bridge, first forming $B_{III}$ offspring via apomeiosis, then resulting in tetraploids via haploid pollen (Schinkel *et al.*, 2017). Similar pathways have been reported from *Paspalum* (Ortiz *et al.*, 2013). A genetic model for evolution of gametophytic apomixis in autopolyploids was proposed for Lactuceae (Asteraceae), based on studies in *Pilosella* and *Hieracium* (Bicknell *et al.*, 2023). First, an insertional mutation, mediated via transposable elements at the avoidance of fertilization (LOP) locus, would lead to parthenogenesis, resulting in haploid offspring. These plants would be of low fertility and viability, but their offspring could be 'rescued' via a second mutation for apomeiosis. Polyploidization would then allow inheritance of the dominant parthenogenesis genetic factor via diploid pollen in the heterozygous state. The combination of mutations in a locus controlling apomeiosis (avoidance of meiosis, LOA) with one in a locus controlling parthenogenesis (LOP) and one for autonomous endosperm formation (AutE) results in fully functional tetraploid apomicts (Bicknell *et al.*, 2023). Also in this model, polyploidization appears to be a secondary factor that might enhance the establishment of the newly formed apomicts (Hojsgaard and Hörandl, 2019).

For sporophytic apomixis, the genus *Citrus* is best studied with respect to genetic control mechanisms. A dominant genomic region in the RWP-RK domain that controls cell fate in plants appears to control nucellar embryony in mandarin, and the gene *CitRWP* was found to be a candidate gene (Wang *et al.*, 2017). *CitRWP* contains a MITE (Miniature Inverted-repeat Transposable Element) inserted into the promoter region of the *CitRWP* gene (Xu *et al.*, 2021). This gene is required to enable nucellar embryo development but is not essential for zygotic embryogenesis. This mutation might have occurred first in mandarin (*Citrus reticulata*), and this species could have transferred the mutation via pollen to other *Citrus* taxa via hybridization, resulting in the apomictic hybrids *Citrus sinensis* and *Citrus aurantium*. These two taxa transferred the mutation further as pollen donors to *Citrus paradisi* and *Citrus limon*, resulting in nucellar embryony in all four taxa (Xu *et al.*, 2021). A similar molecular mechanism was found for *Fortunella* (kumquat), but apomixis originated in Citrinae multiple times (Wang *et al.*, 2022). Plants with nucellar embryony also show elevated expression of auxin transporter and auxin-responsive genes (Xu *et al.*, 2021). It appears that only one dominant mutation is necessary for establishment of sporophytic apomixis, which might explain the higher frequencies of this developmental pathway in angiosperms in comparison to apospory and diplospory (Hojsgaard *et al.*, 2014*a*).

Infectious origins of gametophytic apomixis are possible via pollen, which can pollinate sexual plants and easily transfer apomictic genetic factors to their offspring (Mau *et al.*, 2021). This was documented for diploid apomicts, such as in *Boechera* (Mau *et al.*, 2021), which is rather the exception. In *Rubus*, tetraploid apomictic pollen donors can fertilize diploid and tetraploid sexual species, thereby creating new apomictic genotypes (Šarhanová *et al.*, 2017). In systems with diploid sexuals and polyploid apomicts, different ploidy levels of pollen (e.g. 2*n* from a tetraploid pollen donor) fertilizing reduced egg cells (*n*) will be hampered by a triploid block with strongly reduced seed set (e.g. Ortiz *et al.*, 2013; Mitsuyuki *et al.*, 2014). This effect will be even stronger with greater differences in ploidy levels of parental individuals and can also be blocked by mentor effects, i.e. by induced self-pollination of the diploid mother plant (Hörandl and Temsch, 2009). In experimental crosses of *Taraxacum* of triploid pollen donors and diploid sexual mothers, the seed set was much reduced, but most of the offspring were sexual, resulting from induced selfing; the remaining offspring were mostly triploid, resulting from unreduced pollen, and were only parly apomictic (Tas and Van Dijk, 1999; Van Dijk *et al.*, 1999; Mártonfiová *et al.*, 2007). Results suggest that infectious origins work well only in crosses between the same ploidy levels, e.g. a tetraploid apomictic pollen donor and a tetraploid sexual mother plant. Another factor negatively affecting infectious origins is poor pollen quality of the apomictic pollen donor, which will allow for a long-term coexistence of sexuals and asexuals instead of disappearance of the former according to theoretical models (Mogie, 2011). In natural mixed populations of *Taraxacum*, it was confirmed that pollination by apomicts is not successful enough to replace diploid sexuals unless a single sexual individual is surrounded by apomictic plants

(Martonfiová, 2015). The extent of such infectious origins in natural populations is not well studied in other genera.

### *The role of polyploidy in shifts to apomixis*

Previous hypotheses suggested that polyploidy would be the functional trigger for apomixis by impeding a genomic shock and inducing ectopic or asynchronous gene expression in the genes controlling sexual development (Carman, 1997; Koltunow and Grossniklaus, 2003). Although detailed developmental studies reported asynchrony and changes in expression patterns between apomictic and sexual plants (Sharbel *et al.*, 2010; Hojsgaard *et al.*, 2014*a*), it remains unclear why so few polyploid angiosperms would shift to apomixis. The great majority of polyploid angiosperms remain obligately sexual. The question is whether environmental triggers, genetic or epigenetic changes, or a combination of both is required to undergo a shift to apomixis.

Numerous studies suggest that sexuality and gametophytic apomixis respond differentially to environmental stress conditions, with sex being triggered by increased stress (Schmidt, 2020; Soliman *et al.*, 2021; Niccolò *et al.*, 2023). In plants, this effect is probably attributable to increased oxidative stress in reproductive tissues, initiating meiosis (Hörandl and Hadacek, 2013). In experiments that exposed premeiotic pistils of otherwise sexual species to oxidative stress-reducing treatments (media with antioxidants, glucose, abscisic acid, fluridone and 5-azacytidine), apomeiosis was initiated spontaneously in all species (Mateo de Arias *et al.*, 2020). These observations are in line with results indicating that environmental stress and its metabolic control have effects on the formation of apomeiotic vs. meiotic ovules, but not on parthenogenesis (see above). In natural populations, a reduction of intracellular oxidative stress would consequently initiate apomixis. Here, a potential side effect of polyploidy comes into play (Hörandl and Hadacek, 2013). Polyploidy has been shown to buffer cellular stress factors in plants (Schoenfelder and Fox, 2015; Fox *et al.*, 2020; Van de Peer *et al.*, 2021). Hence, polyploid plants could better retain redox homeostasis and would express apomeiosis more easily. This hypothesis was confirmed by experiments exposing diploid, tetraploid and hexaploid plants of the *R. auricomus* complex to prolonged photoperiods, which increased frequencies of megasporogenesis against apospory in all cytotypes, with the strongest effects in diploids (Ulum *et al.*, 2020). In these experiments, polyploids turned out to be better quenchers of excess light energy and excited electrons, thereby reducing formation of potential reactive oxygen species (Ulum *et al.*, 2021).

However, the question remains whether the environmental stimulus alone would suffice to stimulate the shift from sexual to apomictic seed formation. So far, an environmental influence has been observed only on expression of meiosis/apomeiosis, but not on parthenogenesis or on sporophytic apomixis. And, after all, most polyploid plants do remain sexual. Polyploidy could have positive side effects on establishment of newly evolved apomicts, such as via breakdown of self-compatibility and the shift to uniparental reproduction or by a better ability to colonize stressful environments (Hojsgaard and Hörandl, 2019). These factors would explain the prevalence of polyploidy in apomictic plants without being directly involved in the control of apomixis. For this, a combination of intrinsic factors (i.e. genetic and epigenetic changes in genes regulating reproduction) and certain environmental conditions might be needed to establish functional apomixis (Niccolò *et al.*, 2023). This peculiar combination of intrinsic and extrinsic factors required for a successful shift from sex to apomixis would constrain the origin of apomixis to be a rare event.

## A PHYLOGENETIC MODEL FOR THE EVOLUTION OF SEXUAL REPRODUCTION IN LAND PLANTS

Lewis (1987) emphasized that costs of sex and selective forces for and against sex are not distributed equally among eukaryotes. To understand the main selective forces acting on photosynthetic organisms and in the different clades of land plants, it is also interesting to look into these forces within a phylogenetic framework of eukaryotes, focusing on organisms with complex multicellularity (Bowman *et al.*, 2016) and on the archaeplastid clade (Adl *et al.*, 2019). Meiosis had started in the last common ancestor of eukaryotes, probably as a response to increased intracellular oxidative stress (Fig. 3A; Speijer *et al.*, 2015; Hörandl and Speijer, 2018). The degree of oxidative stress increased drastically with the additional endosymbiosis of a cyanobacterium in the ancestor of the archaeplastid clade and establishment of photosynthesis as an additional intracellular oxidative stress, which probably resulted in the establishment of regular meiosis–fertilization cycles (Hörandl and Speijer, 2018). The next major step increasing stress was terrestrialization, which involves major abiotic stress factors: stronger ultraviolet irradiance than in water and the risk of photo-oxidative stress; drought; larger temperature differences between day and night and between seasons, eventually even with freezing; and increased salinity (de Vries and Archibald, 2018; Fürst-Jansen *et al.*, 2020). Some adaptive features and the genetic toolkit of land plants were already present in streptophyte algae and were combined in embryophytes in a complex and mosaic-like manner (de Vries and Archibald, 2018). The combination of stress factors, especially of photosynthetic stress and photorespiration, probably led to sessility of land plants, because one organism could not keep the redox balance with respiration, photosynthesis and motility as an additional oxidative stress factor (Hörandl and Hadacek, 2013). Concomitant with terrestrialization was the shift to predominant sexuality, whereas asexual reproductive cycles became rare (leaving aside vegetative propagation). The establishment of a diplohapontic life cycle was an advantage for terrestrialization, because cell organization in the diplontic sporophyte allows for an upward organization in the embryo and a better stress response via cell differentiation factors (e.g. evolution of stomata and guard cells for improved regulation of water loss; Fürst-Jansen *et al.*, 2020). In bryophytes, lycophytes and ferns, however, sexual reproduction is still dependent on external water for the fertilization process, because motile spermatozoids need a liquid medium in which to swim to archegonia and egg cells. Seed plants overcame this problem and finally became the only group of land plants successfully able to colonize extremely dry and light-exposed habitats, i.e. deserts, but also cold areas where freezing makes water immobile (some exceptions exist, e.g. *Selaginella*

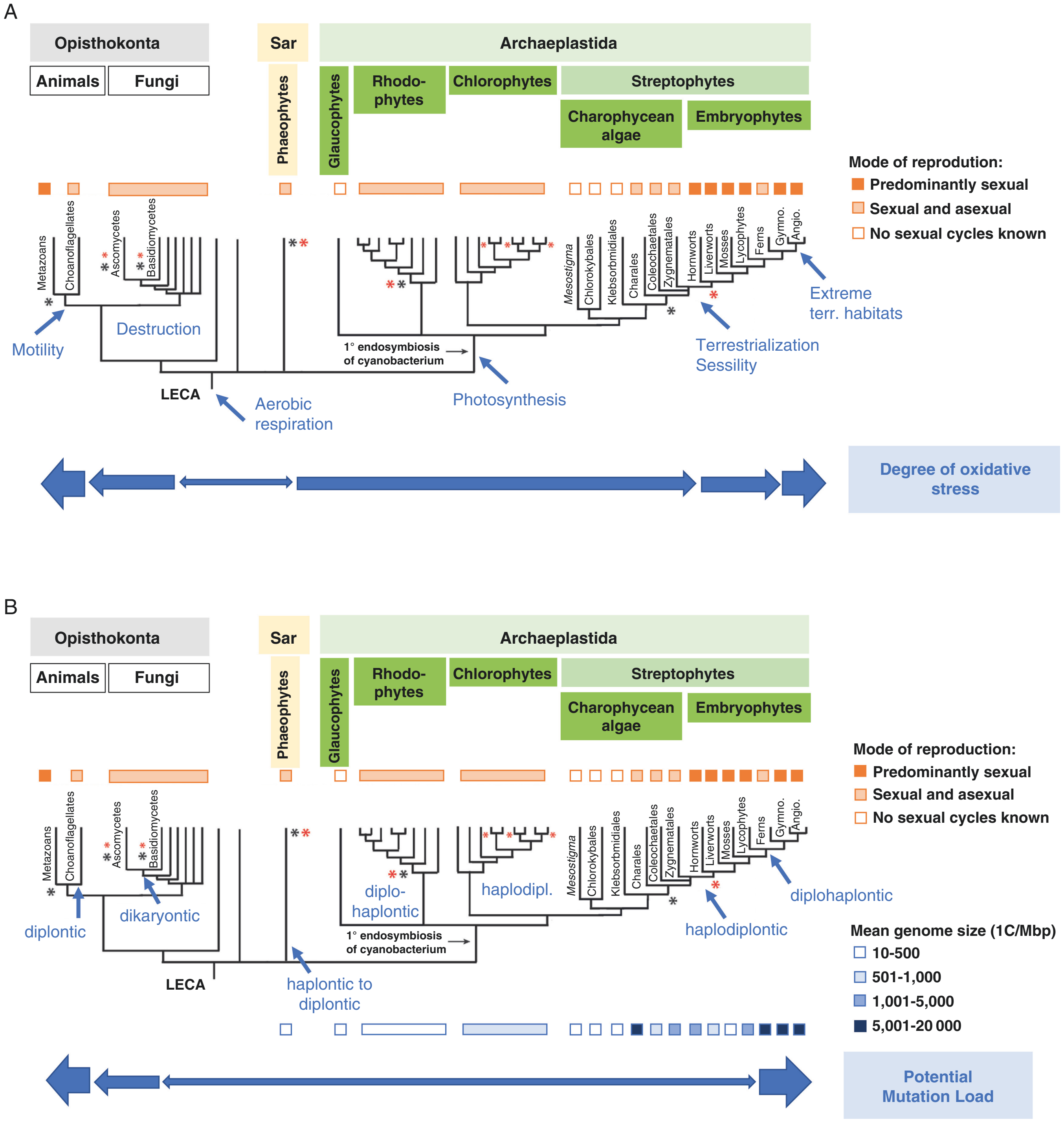

FIG. 3. Simplified phylogenetic tree of eukaryotes depicting lineages resulting in complex multicellularity, i.e. with cell and tissue differentiation (black asterisks). Life cycles with alternation of generations are indicated by red asterisks. Tree topology, multicellularity and life cycles are redrawn after Bowman *et al.* (2016), with the phylogeny of the archaeplastid clade after One Thousand Plant Transcriptomes Initiative (2019) and Wong *et al.* (2020). Mode of reproduction excludes vegetative propagation. Categories: predominantly sexual, >90 % of species; sexual and asexual, both modes in 10–100 % of species; no sexual cycles known, 0 % sex; estimates after Liu *et al.* (2017) for algae and Mogie (1992) for land plants. (A) Hypothetical degree of oxidative stress (blue thick arrows) accumulating over the phylogeny of plants, starting with oxygen respiration in the last common ancestor of eukaryotes (LECA), with additional stress via photosynthesis and a further increase with terrestrialization (de Vries and Archibald, 2018; for comparison with animals and fungi, see Hörandl and Hadacek, 2020). Seed plants colonize terrestrial habitats with extreme abiotic conditions (see main text). (B) Potential mutational load (blue thick arrows), regarding life cycles (reduced haploid selection in diplontic and diplohaplontic life cycles) and average genome size within Archaeplastida (higher mutational load expected in large genomes). Genome size was calculated as mean values for the respective taxa from data in the study by Pellicer and Leitch (2020); the C-values database was accessed on 27 October 2023 (see database for all values and minimum–maximum ranges) and Price *et al.* (2019) for glaucophytes.

*lepidophylla*, a poikilohydric lycophyte growing in deserts). This stepwise increase of stress factors during terrestrialization is roughly concomitant with decreased frequencies of asexual reproduction of land plants in comparison to streptophyte algae (Fig. 3A).

The second major factor in the DNA restoration theory is purging the mutational load. Mutation rates are influenced by multicellularity, increasing genome size, and life cycles (Fig. 3B). Focusing on multicellular organisms with cell differentiation, ploidy cycling via meiosis–fertilization is efficient for elimination of mutations at the haplontic stage. This haploid selection is important specifically in photosynthetic lineages tending to prolonged diplontic phases, such as in the rhodophytes (with a triphasic life cycle), phaeophytes and embryophytes (Fig. 3B). In embryophytes, the increase of genome size comes together with terrestrialization and the shift to dominance of the sporophyte, establishing a diplohaplontic life cycle in vascular plants (lycopods, ferns and seed plants). Consequently, the mutation rate and the threat of accumulation of mutations is expected to be highest in vascular plants. Concomitantly, sexual reproduction is predominant in this clade (Fig. 2B). The relatively high frequencies of asexual reproduction without ploidy cycling in ferns could be a consequence of frequent polyploidization, which allows for a buffering of recessive deleterious mutations over long time periods.

This phylogenetic overview supports a hypothesis that accumulation of intrinsic and extrinsic stress and mutagenesis selectively favoured sexual reproduction in plants. In angiosperms, apomixis appears only with certain combinations of factors and is probably not a long-term evolutionary strategy.

## CONCLUSIONS AND OUTLOOK

This review reveals that the DNA restoration theory is best applicable to plants to explain maintenance of sex. Focusing on shared features of sexual reproduction of all eukaryotes, the functions of meiosis for DNA repair and genetic recombination appear to be universal. The ploidy cycling in plants during meiosis–syngamy cycles is highly efficient in purging deleterious mutations via haploid selection in the prolonged multicellular gametophyte phase. In contrast to animals, the costs of sexes play only a minor role because of predominant hermaphroditism; the cost of mating is rather a cost of pollen transfer from one individual to the other, which has been optimized in angiosperms by adaptations of flowers to either wind or animal dispersal (or both).

Experimental work, transcriptome and genome analyses support the hypothesis of sex being a response to biotic and abiotic stress, mediated by oxidative stress and formation of reactive oxygen species in the tissues. The phylogeny of the archaeplastid clade supports this idea of a stepwise accumulation of endogenous and exogenous stress factors accumulating in land plants and finally in angiosperms, with concomitant high frequencies of obligate sexual reproduction. Alternative asexual developmental pathways appear mostly in polyploids, in which stresses are better buffered; however, concomitant genetic and epigenetic alterations of control mechanisms of reproductive pathways appear to be necessary to establish fully functional asexual reproduction. Such combinations become rare but are realized in ~2.3 % of the genera of angiosperms.

Future research is needed to fill knowledge gaps in the occurrence of apomixis in ferns and angiosperms; many species have never been analysed with respect to their mode of reproduction. We also need more information on the consequences of different developmental sexual vs. asexual pathways on DNA restoration and integrity of the genome. Empirical studies on the function of meiosis as a response to oxidative stress in sexuals and asexuals are needed and should also be done on female meiosis as the more relevant pathway for expression of apomixis. The role of facultative selfing in genome evolution of angiosperms is poorly understood. Whole-genome sequencing of related sexual and apomictic taxa, with consideration of different ploidy levels, is promising to evidence the postulated mechanism of purifying selection against deleterious mutations and diversifying selection for beneficial mutations. The role of polyploidy for establishment of asexual lineages needs to be investigated. Finally, phylogenetic analyses can shed led light on the evolution of sexual reproduction and the constraints for the rare shifts to asexual reproduction in plants.

## SUPPLEMENTARY DATA

Supplementary data are available at *Annals of Botany* online and consist of the following.

Table S1: occurrences of apomixis in angiosperm genera.

## FUNDING

This project was funded by the Deutsche Forschungsgemeinschaft (DFG), grant number HO 4395/10-2 to E.H., within the priority program 'Taxon-Omics: New Approaches for Discovering and Naming Biodiversity' (SPP 1991).

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