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# Chapter 7

## Genome Evolution of Asexual Organisms and the Paradox of Sex in Eukaryotes



**Elvira Hörandl, Jens Bast, Alexander Brandt, Stefan Scheu, Christoph Bleidorn, Mathilde Cordellier, Minou Nowrousian, Dominik Begerow, Anja Sturm, Koen Verhoeven, Jens Boenigk, Thomas Friedl, and Micah Dunthorn**

**Abstract** The predominance of sex in eukaryotes is still enigmatic. Sex, a composed process of meiosis and mixis cycles, confers high costs but the selective advantages remain unclear. In this review, we focus on potentially detrimental effects of asexuality on genome evolution. Theory predicts that asexual lineages should suffer from lack of meiotic DNA repair, accumulation of deleterious mutations, proliferation

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of transposable elements, among others. Here, we compare the different genomic features, life cycles, developmental pathways, and cytological mechanisms in the major eukaryotic groups, i.e., in protists, animals, fungi, and plants. In general, it is difficult to disentangle lineage-specific features from general features of asexuality. In all groups, forms of asexuality are predominantly facultative or cyclical. A variety of mixed or partial sexual developmental pathways exists, maintaining some components of sexuality, while obligate asexuality appears to be rare in eukaryotes. The strongest theoretical prediction for negative consequences of asexuality is decreased effectiveness of selection compared to sexuality. While some studies have shown increased rates of mutation accumulation in asexuals, others using whole-genome comparisons did not find this pattern. Various mechanisms exist that can alleviate the negative consequences of accumulation of negative mutations. More empirical data are needed to understand comprehensively the role of genome evolution for the maintenance of sex.

## 7.1 Introduction

It is still a core question of evolutionary biology why sexual reproduction is so predominant in eukaryotes (Otto 2009; Neiman et al. 2018). Sex can be broadly defined as “a process in which the genomes of two parents are brought together in a common cytoplasm to produce progeny which may then contain reassorted portions of the parental genomes” (Birdsell and Wills 2003). In eukaryotes, sex is a composite process consisting of meiosis, as a special form of nuclear division, and fertilization

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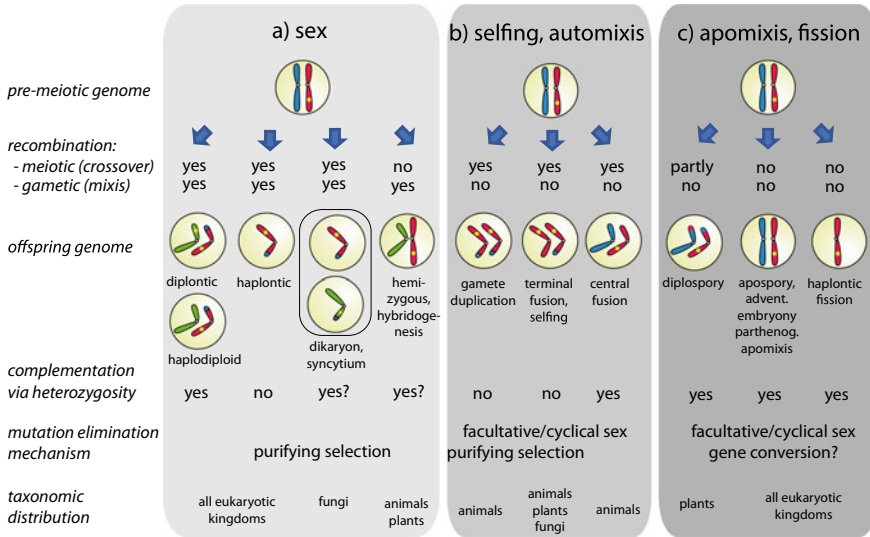
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with fusion of nuclei (syngamy and karyogamy; Birdsell and Wills 2003; Brandeis 2018). Both components, however, imply high costs for the parental organisms: meiosis involves a breakup of favorable gene combinations and is altogether a risky, energy-consuming process. Syngamy and karyogamy (outcrossing) involve the need of two mating partners, and hence the costs of mate searching, mate finding, density dependence, eventually a cost of non-reproducing males, among others (Maynard Smith 1978; Bell 1982); these costs differ dramatically among eukaryotic taxa (Lewis 1987; Hörandl and Hadacek 2020). In general, sexual reproduction has no immediate selective advantage for the individuals performing sex. Hence, it is difficult to explain maintenance of the process and also the prevalence of obligate sexuality. Because of its high costs, sex should theoretically be replaced by asexuality (Williams 1975; Maynard Smith 1978; Bell 1982; Lewis 1987; Otto 2009). Prokaryotes demonstrate that organisms can be evolutionarily successful and diverse with asexuality, they can adapt to almost all ecological niches of this planet, and they are much older than eukaryotes—all without the burdens of meiosis–misis cycles.

Sexual reproduction probably originated already in the first eukaryotes (2010; Speijer et al. 2015; Hörandl and Speijer 2018) and had its precursors in prokaryotes (Birdsell and Wills 2003; Ramesh et al. 2005; Speijer et al. 2015; Speijer 2016). We will focus here on maintenance of sex in eukaryotes. While sexuality is still infrequent in protists, it becomes increasingly more frequent in multicellular organisms where sex and reproduction are intimately linked and reaches dominance in animals and flowering plants, with >99% of sexual species in both groups (Burt 2000). A great diversity of adaptations has evolved in plants and animals to make sexuality and mating between conspecific individuals working (Brandeis 2018), but the genetic control of meiosis (Schurko and Logsdon 2008) and the basic nuclear and cellular processes in meiosis–misis cycles have remained surprisingly conserved.

By contrast, asexuality evolved multiple times from sexual ancestors (Schwander and Crespi 2009a, b). Asexuality occurs in eukaryotes in many different cytological and developmental pathways (Fig. 7.1, Box 7.1), whereby most of them represent just various alterations of meiosis–misis cycles (Mirzaghaderi and Hörandl 2016). Sexual reproduction is hardly ever abandoned completely—in fact, “a little bit of sex,” i.e., facultative or cyclical asexuality appears to be common in fungi, plants, and animals (Simon et al. 2003; Mirzaghaderi and Hörandl 2016). The wealth of asexual cytological and reproductive pathways in eukaryotes and the nightmare of different terminologies in the traditional literature (see Schön et al. 2009) have hampered comparative empirical research (see Fig. 7.1, Box 7.1). Our knowledge on asexual genome evolution is restricted to a handful of organisms, with a strong focus on metazoans (Neiman et al. 2018). These biases made it difficult to develop generalized models of advantages of sexuality over asexuality.



**Fig. 7.1** Overview of cytological mechanisms in major sexual and asexual developmental pathways, and main consequences for genome evolution. For terminology, see Box 7.1. The yellow dots represent disadvantageous mutations that are either complemented by unmutated homologous chromosomes or are selectively eliminated by various mechanisms. **a** Sexual reproduction, with meiosis and mixis, i.e., biparental genome contributions (symbolized by green chromosome). **b** Reproduction with meiosis, but self-fertilization and hence uniparental genome contributions. **c** Reproduction without meiosis and without mixis

### Box 7.1. Forms of Asexual Reproduction and Definition of Terms

**Adventitious embryony:** a form of apomixis in plants whereby embryos develop directly out of a somatic cell.

**Apogamy:** a term for apomixis in ferns.

**Apomictic parthenogenesis:** the term for apomixis in animals. See also thelytoky.

**Apomixis:** asexual reproduction without meiosis and with parthenogenesis, usually applied to multicellular organisms. Offspring are clones of the parent.

**Apospory:** a form of apomixis in plants whereby egg cells develop from a somatic cell and develop parthenogenetically.

**Automixis:** involves meiosis and fusion of nuclei derived from the same meiosis, but nuclei originate from the same parental individual. Eggs develop parthenogenetically. Occurs mostly in animals and is usually regarded as a form of asexual reproduction.

**Central fusion:** a form of automixis involving fusion of segregated nuclei derived from meiosis I. Maintains mostly the parental heterozygosity.

**Cyclical parthenogenesis:** Life cycle comprising an alternation of sexual and asexual reproduction (many animals).

**Dikaryon:** cells that have two haploid nuclei in their major life stage (occurs in mycelia of some fungi).

**Diplospory:** a form of apomixis in plants involving a restitutional meiosis, but parthenogenetic development of egg cells.

**Facultative apomixis:** sexual and apomictic offspring are produced in parallel in one life cycle, from the same mother (frequent in plants).

**Fission:** Mitotic cell divisions. Usually applied to unicellular eukaryotes (e.g., fission yeast).

**Gamete duplication:** a form of automixis whereby chromosome sets duplicate after meiosis II. Results in complete homozygosity.

**Gynogenesis:** the male gamete is necessary for development, but is not included in genome of offspring. Offspring is formed via apomixis or automixis. Occurs in some e.g., vertebrates.

**Haplodiploidy:** Females are diploid and produce meiotically haploid eggs. Fertilized eggs become diploid females, unfertilized eggs become haploid, parthenogenetic males (occurs in insects) also called arrhenotoky. Haplodiploidy can evolve into parthenogenetic apomixis.

**Hemizygous reproduction:** the maternal genome is transmitted without recombination, while the paternal genome is only partially inherited (e.g., canina meiosis in dogroses) or not inherited (see hybridogenesis).

**Homothallic selfing:** two nuclei derived from a meiosis of the same individual (the same mating type) fuse. Occurs in fungi.

**Hybridogenesis:** a form of hemizygous reproduction whereby female genomes are inherited clonally, while male genomes are continuously recruited for one generation, but not inherited (occurs in e.g., vertebrates; see also gynogenesis).

**Parthenogenesis:** development of an egg or egg cell into an embryo without fertilization. Occurs in plants and animals.

**Pseudogamy:** a male genome contribution is needed for development of embryos, but is not inherited. Occurs in animals and plants (but with different mechanisms).

**Selfing (self-fertilization, autogamy)** involves fusion of nuclei derived from two meioses. Gametes originate from the same individual. Occurs frequently in flowering plants (also called autogamy) but is rare in animals. Selfing is in plants usually regarded a form of sexual reproduction, but cytologically more similar to automixis in animals. See also homothallic selfing.

**Sexual reproduction (amphimixis, outcrossing):** involves meiosis (consequently recombination via crossovers) and mixis (syngamy and fusion of nuclei from different individuals, and hence gametic recombination). The life cycle can be diplontic (predominant diploid stage) or haplontic (predominant haploid stage).

**Syncytium:** cells with many nuclei that may have different origin (occurs in some fungi).

Terminal fusion: a form of automixis whereby sister nuclei derived from meiosis II fuse, resulting in complete homozygosity. Occurs in animals.

Thelytoky (thelytokous parthenogenesis): females produce female offspring from unfertilized eggs. The cytological mechanism can be automictic or apomictic. Occurs in animals.

Translocation heterozygosity: a form of meiosis with ring-forming chromosomes, without recombination, combined to selfing. Occurs in a few plants.

About 20 hypotheses exist for explaining the maintenance of sex, and they can be grouped into two major theories (Williams 1975; Kondrashov 1993; Birdsell and Wills 2003): first, sex is a tool for DNA restoration to keep the integrity and functionality of the nuclear genome, whereby most mechanisms have long-term effects (Muller 1964; Bernstein et al. 1988; Holliday 1984; Hörandl 2009); second, sex and recombination create variation in the offspring for a better short-term response and adaptive potential to environmental variability (Weismann 1904; Maynard Smith 1978).

Long-term benefits of sex act at the lineage level and unfold only after many successive generations. They derive, for example, from the ability to effectively combine beneficial alleles arising in different lineages (Fisher–Muller hypothesis; Fisher 1930; Muller 1932); from restoration of genotypes least loaded with deleterious mutations that are continuously lost by drift in finite populations (Muller's ratchet; Muller 1964), from removal of linkage between beneficial and deleterious alleles (Hill and Robertson 1966), or from enabling repair of deleterious alleles via meiotic gene conversion (Bernstein et al. 1988; Marais 2003). Hypotheses on the selective advantage of sex inherently predict that asexuality comes with specific disadvantages that manifest themselves in the genome over evolutionary time scales.

Short-term benefits of sex unfold under strong fluctuating directional selection at the level of individuals and genes. Furthermore, individual selection allows sexual populations to withstand succumbing to the cost of sex, e.g., via quick replacement by asexual offshoots. The rationale is that sex enables effective selection by generating variation in fitness through break-up of linked loci with opposite fitness effects (Felsenstein 1974). Such negative linkage disequilibria can be generated via combined effects of drift and selection in finite populations (Hill–Robertson interference; Hill and Robertson 1966). Thus, for sex to be beneficial in the short term, the trajectory of selection must change constantly (Brooks 1988); e.g., through coevolving parasites (fluctuating epistasis, Red-Queen; reviewed in Jaenike 1978; Hamilton 1980) or spatial variation in the availability of resources coupled with abiotic conditions (Scheu and Drossel 2007; Song et al. 2011). A constant change of selection scenarios, however, is an unrealistic assumption, and hence an insufficient explanation for the maintenance of obligate sexuality. Theories and empirical studies on the maintenance of sex have been recently reviewed by Jalvingh et al. (2016) and Neiman et al. (2018).

These two theories are not mutually exclusive, and also pluralistic approaches combining both ideas have been proposed (West et al., 1999; Neiman et al. 2017). Here, we will focus on theories regarding genome evolution, because the universality of the genetic code is comparable among all eukaryotes. Moreover, methodological advances of genome sequencing in the last two decades have brought upon new empirical data for genome evolution in eukaryotes and have shed a new light on old theories. First, we will review the most important theories about the role of sex in maintaining genomic integrity; second, we will report the state of the art on asexual genome evolution over all major groups of eukaryotes, including protists, algae, animals, fungi, and plants. Finally, we will draw preliminary conclusions and outline perspectives for future research.

## 7.2 The Theoretical Background for Asexual Genome Evolution

Several authors have argued that a major function of sex is maintaining genomic integrity (Muller 1964; Holliday 1984; Bernstein et al. 1988; summarized in Birdsell and Wills 2003; Hörandl 2009). Sex as tool for DNA restoration involves three different components: first, DNA repair, such as repair of physical DNA damage on the molecular structure of DNA (Bernstein and Bernstein 1991); second, elimination of deleterious mutations, i.e., changes in the sequence of base pairs); and third, maintenance of DNA methylation. These three components may act in combination (Hörandl 2009).

### 7.2.1 DNA Repair

The need for DNA repair is a constant and immediate pressure for all organisms. Transformation, a process through which prokaryotes are able to uptake exogenous DNA, is a probable precursor of sex. In prokaryotes, this mechanism is used for physical DNA repair, as prokaryotes needed to cope with DNA breaks caused by UV irradiation, temperature extremes, and other sources of oxidative damage (Birdsell and Wills 2003). In eukaryotes, many enzymes for homologous recombinational repair evolved into meiosis genes or became integrated in the eukaryotic meiosis machinery (Ramesh et al. 2005; Malik et al. 2008; Schurko and Logsdon 2008). Bernstein et al. (1988) and Bernstein and Bernstein (1991) recognized that the cytological processes during prophase I of meiosis are mainly directed to repair DNA double-strand breaks, while recombination resulting from cross-overs appears to be an infrequent by-product of this process. Meiosis research has so far confirmed these findings (reviewed by Mirzaghaderi and Hörandl 2016). Eukaryotic cells may have



evolved advanced DNA repair mechanisms because of increased intracellular oxidative stress caused by aerobic respiration and other redox-active metabolic processes that have increased damage of DNA. The evolution of linear chromosomes, of the nuclear envelope and of homologous recombinational repair at meiosis I, could have been driven by this selective force (Speijer et al. 2015; Hörandl and Speijer 2018). Homologous recombination is the most accurate and least mutagenic repair mechanism of DNA breaks (Bleuyard et al. 2006), but it requires a second homologous chromosome as a template. This requirement enforced a diploid stage in the life cycle and a chromosome from another individual with a different history of damage. The requirement of diploidy could have been the major driver for the evolution of mixis (Hörandl and Speijer 2018). In modern meiosis, minor DNA lesions, i.e., DNA radicals caused by oxidative damage, could initiate meiotic homologous recombinational DNA repair (Hörandl and Hadacek 2013).

### 7.2.2 *Mutation Accumulation*

Inaccurate repair of DNA damage is a major source of mutagenesis (Friedberg et al. 2006). Non-homologous repair mechanisms are prone to mutagenesis, while homologous recombinational repair, acting during meiosis, is the least mutagenic mechanism (Bleuyard et al. 2006). Most mutations are either neutral or have negative effects, and deleterious mutations are a constant threat for functionality of genomes. Mutations (changes of the sequence of DNA bases) cannot be actively repaired; they can only be eliminated or favored by selection according to their effect on the fitness of the organism (Bernstein et al. 1988). The theory of Muller's ratchet predicts that without sex and recombination, deleterious mutations would accumulate in a lineage over generations in finite populations (Muller 1964; Kondrashov 1988; Charlesworth et al. 1993a, b). Recombination can reinstall non-mutated genomes in the offspring, whereas in an asexual lineage, the class of non-loaded or least-loaded class of individuals will get lost by drift. This irreversible process represents a click of the ratchet. Hence, in asexual lineages, mutations will accumulate gradually over generations until a certain threshold of deleterious mutations is reached when the lineage goes extinct.

Muller's ratchet clicks at a constant rate depending on population size ( $N$ ), the deleterious mutation rate per haploid genome ( $U$ ), and the strength of selection ( $s$ ) (e.g., Jain 2008). Infinite populations are not affected by Muller's ratchet as a class of non-mutated individuals will always be present, while in finite populations the least-loaded class will be more easily lost by drift (Birdsell and Wills 2003). The deleterious mutation rate of course differs between eukaryotic organisms and has been empirically tested for just a few model organisms. For diploid or polyploid organisms, the mutation rate  $U$  multiplies with the ploidy level  $c$  (Gerstein and Otto 2009). In diploid or polyploid genomes, or in fungal dikaryons, mutations appear in the heterozygous state, and functional alleles may "mask" the recessive mutated gene copy from selection (Kondrashov and Crow 1991; Gerstein and Otto 2009).

Hence, mutation accumulation should have little effect in the short term, but may be in the long term more severe in polyploids than in diploids, because mutations are eliminated slower than in diploid lineages (Gerstein and Otto 2009). The actual speed of the ratchet depends further on recombination rates (Charlesworth et al. 1993a, b), epistasis between genes (Kondrashov 1988), and effects of beneficial mutations (Muller 1932; Crow and Kimura 1965). Residual sexuality with a little bit of recombination is sufficient to halt Muller's ratchet (Green and Noakes 1995; Hodac et al. 2019). The deterministic mutation model by Kondrashov (1988) involves synergistic epistasis between deleterious mutations such that their combined effect is more severe than the sum of their individual effects. Truncating selection is assumed to act on individuals that carry many such synergistic mutations, and hence the death of these individuals will eliminate many mutations from the population. Sex is here of great advantage as recombination breaks up linkage disequilibrium, i.e., the negative gene combinations, and increases the variance on which selection can act upon. However, empirical evidence rather suggested that negative epistasis between deleterious mutations is uncommon (Kouyos et al. 2007). Epistasis appears under the precondition of pleiotropy and evolves in a dynamic manner, depending on robustness and complexity of genomes (de Visser and Elena 2007; de Visser et al. 2011). Finally, the rare beneficial mutations have to be considered as large sexual populations can incorporate them more rapidly than asexual ones, whereas in small populations, there would be no difference between sexual and asexual populations in the speed of incorporating beneficial mutations (Fisher 1930; Muller 1932).

For prokaryotes, mutation accumulation via Muller's ratchet is a lesser problem because of large population size, rapid generation turnover, and small, haploid genomes. Prokaryotes further avoid mutation accumulation via frequent lateral gene transfer from one lineage to the other, which is under strong purifying selection (Vos et al. 2015). In eukaryotes, however, the parameters determining genome evolution become more diverse and more complex. The diversity of life cycles, mutation rates, population sizes, the more complex organization of genomes with manifold more genes, and the diversity of reproductive systems with varying recombination rates makes it difficult to apply one model that fits all organisms. While theoretical studies support the idea that mutation elimination is a strong advantage of sex, empirical research on various organisms gives an equivocal picture (see Sects. 7.3–7.6).

### 7.2.3 *Epigenetic Damage and Transposable Elements*

Holliday (1984) suggested that removal of epigenetic defects in germline cells and reinstallation of lost cytosine methylations would be a major function of meiosis. Similar as for the DNA repair hypothesis, an integer template of a second homologous chromosome is needed for maintenance methylation. Methylations are crucial for cell differentiation, development, and regulation of gene expression and are evolutionary ancient in eukaryotes (Law and Jacobsen 2010). Although DNA methylations are to

some extent heritable (Law and Jacobsen 2010), there still is uncertainty about long-term evolutionary effects. In the context of paradox of sex theories, little attention has been paid to Holliday's theory. Most research on differences of methylation patterns between sexual and asexual organisms has been done in plants (see Sect. 7.6).

Another aspect of genome evolution related to methylation is the proliferation of transposable elements (Hickey 1992). Three hypotheses exist regarding TEs in eukaryotic genomes: (1) TEs could be sexually transmitted and spread like "genomic parasites," and so their spread could be avoided by asexuality (Wright and Finnegan 2001). (2) Purifying selection after meiotic recombination can act against TE proliferation in sexual species. Hence, asexual species may suffer from an uncontrolled proliferation of TEs and may be even driven to extinction (Arkhipova and Meselson 2000, 2005). (3) Finally, there might be no relationship between TE proliferation and mode of reproduction. We will also review new insights into this complex topic in the next paragraphs.

### 7.3 Asexual Genome Evolution in Protists

The diversity of protists is extremely large, i.e., it covers all major eukaryotic clades, except the animals and fungi (Amorphea clade) and plants (Embryophyta clade) (Boenigk et al. 2015; Adl et al. 2019). Although once thought to be proto-animals or proto-plants, protists are now known to form numerous independent lineages that comprise the bulk of the genetic and metabolic diversity within the eukaryotes (Keeling and Burki 2019). Since the origins of eukaryotes sometime in the Proterozoic (Eme et al. 2014), protists have evolved into numerous ecological roles that are central in most ecosystems (Azam et al. 1983; Weisse et al. 2016).

#### 7.3.1 *Sex and Reproduction in Protists*

Although meiotic sex likely originated in the common ancestor of all extant eukaryotes, many protist species and higher clades were long thought to be asexual because of the absence of direct observations of mating between individuals (Schurko et al. 2009; Dunthorn and Katz 2010). On theoretical grounds based on the genetic advantages of recombination, it has been argued that most protists are nevertheless likely cryptically sexual at least occasionally (Dunthorn and Katz 2010; Hofstatter and Lahr 2019). And, on experimental grounds, evidence for this cryptic sex throughout the protists has been found by inventorying meiotic genes in different putative asexual lineages (Ramesh et al. 2005; Malik et al. 2008; Chi et al. 2014b; Dunthorn et al. 2017; Hofstatter et al. 2018; Kraus et al. 2018), although these meiotic genes could be used just for selfing or for non-canonical genetic pathways (Dunthorn et al. 2017). Sex has, however, been lost in some lineages, some of which could be ancient (Doerder 2014).

Meiotic sex in protists was elegantly shown to reverse the effects of Muller's ratchet by Calkins (1919), in what was called "one of the most important experimental results in biology" (Bell 1988). After preventing mating in the ciliate *Uroleptus*, division rates slowed down. Presumably, the slowdown in division rates was due to Muller's ratchet. But after mating, faster division rates were restored and thus, sex was able to "rejuvenate" cultures of ciliates in the laboratory.

Although most protists are likely meiotically sexual and therefore can use sexual recombination to reverse Muller's ratchet, the majority of reproduction in the largely unicellular protists is just mitotic cell divisions (Dunthorn and Katz 2010). Given their small sizes and fast mitotic division times, protists therefore can have massive population sizes (Finlay 2002), although the effective population sizes are smaller (Watts et al. 2013). These massive population sizes could potentially prevent Muller's ratchet by allowing selection to be more powerful than drift, as the rate of loss of few mutations depends on the absolute number of individuals (Bell 1988). Within most natural communities in different environments, though, not all protist species will have these massive population sizes (Dunthorn et al. 2014; Logares et al. 2014), thus the rare species may not necessarily be able to prevent or slow down Muller's ratchet.

### 7.3.2 Protistan Genome Structures and Muller's Ratchet

Genome architecture is known to help drive the strength and direction of evolution (Lynch 2007). There are three aspects to genome structure in protists that may be able to prevent, or at least slow down, Muller's ratchet.

The first aspect is that many protists are highly polyploid (Raikov 1982). For example, even between closely related ciliate species in *Paramecium*, there are multiple rounds of whole-genome duplications (Aury et al. 2006), and extreme levels of gene repetitions are observed in the foraminiferan *Reticulomyxa filosa* (Glöckner et al. 2014). Maciver (2016) suggested that in protists such high levels of polyploidy may help prevent or slow down Muller's ratchet, if deleterious mutations in one of the gene copies are replaced by other copies. This mechanism could be powerful if there is pervasive gene conversion and as long as there is always a gene copy with fewer deleterious mutations. Independent of the evolutionary time or the number of generations, gene conversion would not be successful if all copies had deleterious mutations. The extent of high levels of ploidy level is unknown within most protist species and higher clades, so it is unknown whether polyploidy may prevent or slow down the effects of Muller's ratchet and in how many species such effects occur.

The second aspect is the highly dynamic nuclear genome sizes of many protists (Parfrey et al. 2008). The genome sizes can greatly increase and decrease throughout the life cycle. In the well-known *Amoeba proteus*, this excessive DNA is eliminated late in interphase before mitotic division. Chromatin is ejected during this process

from the nucleus into the cytoplasm, where it is presumably degraded and recycled. Goodkov et al. (2019) suggested that such DNA extrusions may allow protists to prevent or slow down Muller's ratchet, if gene copies with deleterious mutations are being eliminated. This mechanism could be powerful if deleterious mutations are selectively removed, but if removal is random then the most abundant copies will just likely be eliminated. And as with polyploidy above, the extent of elimination of excessive DNA (and the excretion of deleterious gene copies) is unknown within most protist species and higher clades.

The third aspect only occurs in ciliates having two types of nuclei in each cell: micronuclei and macronuclei (Katz 2001; Lynn 2008). Micronuclei are transcriptionally inactive and are involved in sex and the formation of macronuclei. Division of micronuclei occurs through canonical mitosis or meiosis where homologous chromosomes are segregated by a spindle apparatus, although a functional synaptonemal complex is missing (Chi et al. 2014a). Macronuclei are transcriptionally active and are highly polyploid. Division of macronuclei occurs through amitosis, where chromosomes are randomly distributed without a spindle apparatus (Morgens and Cavalcanti 2015; Zhang et al. 2019). This random distribution of chromosomes during amitosis can lead to the loss of gene and chromosome copies in resulting progeny and eventual death if all copies are lost (Bell 1988; Zhang et al. 2019), which is a form of deleterious mutation accumulation. The ciliate *Tetrahymena thermophila* somehow controls chromosome copy number during amitosis, although the mechanism is not clear (Zhang et al. 2019), and some type of similar mechanism is likely found in many more ciliate groups (Morgens and Cavalcanti 2015). If ciliates go through meiosis and then self, however, any gene or chromosome loss, and consequently Muller's ratchet, can be reversed because new macronuclei with the full complement of genes and chromosomes are newly formed by the new micronuclei after selfing unless there were also gene losses in the micronuclei (Bell 1988).

## 7.4 Asexual Genome Evolution in Animals

Obligate asexuality (here female-producing parthenogenesis; thelytoky) is assumed to be rare in animals, found in approximately 0.1% of species (White 1977; Bell 1982). However, this number is based on the very scarce occurrence or even absence (e.g., birds and mammals) of asexuality in vertebrates (White 1977). Recent quantitative studies indicate that obligate asexuality has evolved much more frequently in species-rich non-vertebrate taxa like arthropods and molluscs, for example, with up to 1.5% in haplodiploid arthropod species under conservative estimates (under more relaxed assumptions up to 38% (van der Kooi et al. 2017)). Thus, the occurrence of obligate asexuality in animals seems vastly underestimated and understudied.

The number of parthenogenetic species largely depends on the rate with which incipient asexual lineages are generated and subsequently lost again in an animal group, but very little is known about the frequency of such transitions (Schwander and Crespi 2009a, b). The transition to asexuality from sexual progenitors can be caused

by different mechanisms, such as hybridization, endosymbiont infection, and spontaneous mutations (for an overview see Neiman et al. 2014; Jaron et al. 2019). Moreover, offspring can be generated from unfertilized eggs via many different cellular mechanisms, such as apomixis and automixis with a plethora of diverse subforms (Fig. 7.1; see e.g. Suomalainen et al. 1987; Schön et al. 2009). The underlying mechanisms for both the transition to and cytology of asexuality can have profound and different consequences for genome evolution (Engelstädter 2017; Jaron et al. 2019; Parker et al. 2019), but are, as yet, little studied in animals. By contrast, explaining the short-term and long-term benefits of sex has received considerable attention in both theoretical and empirical studies on animals (Sharp and Otto 2016; Neiman et al. 2017, 2018).

### 7.4.1 Accumulation of Slightly Deleterious Mutations

The Hill–Robertson effect and Muller’s ratchet predict a reduction of the effectiveness of purifying selection resulting in the accumulation of fixed and segregating slightly deleterious mutations in asexual species (see Introduction and, e.g., Keightley and Otto 2006). This prediction received equivocal support in animals: four out of eight available single gene-based studies found less effective purifying selection in asexual as compared to closely related sexual species (for details see Hartfield 2016; Glémin et al. 2019). However, a number of genome-based studies found excessive among gene variation in effectiveness of purifying selection indicating that interpreting single gene-based results as representative for the genome level is problematic (see Neiman et al. 2018). Further, only one (*Timema* stick insects; Bast et al. 2018) out of nine genome-based studies supports less effective purifying selection in asexuals compared to their sexual sister species (Ament-Velásquez et al. 2016; Bast et al. 2018; Brandt et al. 2017, 2019; Kraaijeveld et al. 2016; Lindsey et al. 2018; Ollivier et al. 2012; Tucker et al. 2013; Warren et al. 2018). Notably, two out of these studies based on genomic data even showed increased effectiveness of purifying selection in asexuals, including ancient asexual oribatid mites, contrary to predictions (Kraaijeveld et al. 2016; Brandt et al. 2017).

What factors can alleviate the predicted negative effects allowing asexuals to escape mutational meltdown? Large population sizes have been discussed as an important factor maintaining effective purifying selection under asexuality (Gordo and Charlesworth 2000; Rice and Friberg 2009; Normark and Johnson 2011; Ross et al. 2013). Many widely distributed and small-bodied animals have potentially very large populations (Gaston et al. 1997; White et al. 2007). Indeed, census population densities of very old asexual taxa (e.g., the above-mentioned oribatid mites) can exceed  $10^5$  individuals per square meter and generally feature larger population sizes than their sexual relatives (Maraun et al. 2012). In addition to population sizes, extensive DNA repair and/or homogenizing processes like mitotic gene conversion, or facultative recombination during cyclical parthenogenesis may play an important

role by removing deleterious alleles and exposing recessive deleterious mutations to selection (Charlesworth et al. 1993a, b; Marais 2003).

### 7.4.2 *Accumulation of Deleterious Transposable Elements in Animals*

In non-recombining genome regions of sexual species, deleterious transposable elements (TEs) can rapidly and substantially increase in numbers (e.g., *Drosophila* neo-Y chromosomes; Bachtrog et al. 2008). A number of empirical studies tested whether such accumulation of TEs extends to the genome scale in completely non-recombining genomes of obligate asexual animals, potentially generating selection for sex at the lineage level (similar to the accumulation of point mutations, but possibly more rapidly). No overall genomic difference could be detected between asexual and related sexual animals, only very variable and lineage-specific TE dynamics were found (Bast et al. 2016; Szitenberg et al. 2016; Jiang et al. 2017; Jaron et al. 2019). This lack of difference is likely due to a number of confounding factors not related to reproductive modes (such as, e.g., hybridization and polyploidization) that can affect TE dynamics (Arkhipova and Rodriguez 2013). Despite no overall difference, higher TE turnover in cyclically sexual *Daphnia pulex* indicates that sex facilitates both the spread and elimination of TEs (Jiang et al. 2017). The few investigated older asexual animals harbor few and inactive TEs (Flot et al. 2013; Bast et al. 2016). Whether this stems from the evolution of benign TEs via suppression mechanisms (as indicated in experimentally evolved yeast; Bast et al. 2019; for a review on mechanisms see Koonin et al. 2020) or from the immediate extinction of asexual lineages with high TE contents after the loss of sex remains an open question.

### 7.4.3 *The “Meselson Effect”*

Homologous chromosomes in asexual organisms are expected to accumulate mutations independently of each other in regions sheltered from loss of heterozygosity and diverge in parallel. This should lead to high levels of heterozygosity and parallel topological resemblance of haplotype subtrees over populations (Birky 1996; Judson and Normark 1996; Mark Welch and Meselson 2000). Testing this “Meselson effect” is important because its presence is regarded as strong support for the complete absence of sex and theoretically opens the possibility for dating the transition to asexuality in the absence of fossils (Normark et al. 2003). As yet, only single gene-based studies in asexual *Timema* stick insects and fissiparous *Dugesia* flatworms have shown the expected haplotype divergence pattern (Schwander et al. 2011; Leria et al. 2019). Large within-individual variance levels were found in a number of different

invertebrates, e.g., the apomictic *Meloidogyne* root-knot nematodes and the ribbon worm *Lineus pseudolacteus* but (later) attributed to divergence between homeologs derived from hybridization (Lunt 2008; Ament-Velásquez et al. 2016; Jaron et al. 2019). Similarly, large within-individual variance in bdelloid rotifer species has been shown to result from an ancient genome duplication event resulting in tetraploidy and reflect divergence between ancient homologs (so-called ohnologs) instead of haplotypes (Mark Welch et al. 2008; Flot et al. 2013; Nowell et al. 2018). In other animal species, which show no sign of the Meselson effect, such as darwinulid ostracods or tramini aphids, haplotype divergence has been putatively reduced due to homogenizing processes like mitotic gene conversion (Normark 1999; Schön and Martens 2003). A genome-wide comparison of asexual and sexual lineages of *Daphnia pulex* showed that loss of heterozygosity via such homogenizing processes is a dramatically more powerful force than accumulation of new mutations (Tucker et al. 2013).

#### **7.4.4 Genomic Features Based on Single Asexual Genome Studies**

The genomes of singular asexual animal species featured some peculiarities that were suggested to be generally linked to asexuality, such as horizontal gene transfer (HGT), genomic rearrangements, gene family expansions, gene losses, and gene conversion (Danchin et al. 2010; Flot et al. 2013; Faddeeva-Vakhrusheva et al. 2017; for a full review see Jaron et al. 2019). Many of these features are related to the idea that contrary to sexually reproducing organisms, asexuals do not require chromosomal homolog pairing during meiosis, which potentially leads to increased fixation of structural variants. However, none of the features were systematically replicated across 26 published animal genomes, suggesting that these genomic peculiarities are lineage-specific and not generally linked to asexuality (Jaron et al. 2019). Testing this idea further needs whole-genome studies on structural variants in asexuals compared to closely related sexual species (see outlook).

#### **7.4.5 Evolutionary Scandals: Ancient Asexuals**

Genomic consequences of asexuality with detrimental fitness effects are expected to accumulate over time and eventually drive asexual lineages to extinction (Gabriel et al. 1993; Lynch et al. 1993). However, few asexual lineages have persisted and even diversified in the absence of sex for considerable periods of time (Judson and Normark 1996; Schön et al. 2009; Schwander et al. 2011). The most notorious examples include bdelloid rotifers, darwinulid ostracods, and several parthenogenetic taxa of oribatid mites (Judson and Normark 1996; Schön et al. 2009). Among these, bdelloid rotifers have, so far, received most attention (Mark Welch and Meselson 2000; Flot et al.



2013). Recent studies, however, have indicated that cryptic gene exchange renders them quasi-sexual (Signorovitch et al. 2015; Debortoli et al. 2016; Schwander 2016; Vakhrusheva et al. 2018; Laine et al. 2020). The amount and mechanism of cryptic sex and DNA uptake remain controversial (Flot et al. 2018; Wilson et al. 2018). Data on genome evolution in asexual oribatid mites and darwinulid ostracods are scarce. While for asexual oribatid mites two studies showed effective purifying selection and decreased load of transposable elements (Bast et al. 2016; Brandt et al. 2017), there are currently no genome data-based studies in darwinulid ostracods. More studies on these two animal groups are urgently required as truly ancient asexual lineages are invaluable for generating insights into the long-term selective advantage of sex.

## 7.5 Asexual Genome Evolution in Fungi

Fungi are an ancient, species-rich lineage of eukaryotes with a wide variety of lifestyles (Hawksworth and Lücking 2017; Spatafora et al. 2017). Fungi can be unicellular (yeasts) or multicellular (filamentous fungi); the latter forming cell filaments (hyphae) that form tissue-like networks (mycelia). Fungi can undergo asexual propagation either through mitotic cell division in the case of yeasts, or through hyphal fragmentation or the formation of mitotic spores in the case of filamentous fungi (Golan and Pringle 2017). Sexual propagation in fungi, leading to the formation of meiotic spores, is usually induced under species-specific conditions and can be facultative or an integral part of the life cycle as is the case for a number of plant-pathogenic fungi (Bennett and Turgeon 2016; Peraza-Reyes and Malagnac 2016; Coelho et al. 2017; Lee and Idnurm 2017). While many fungal species were described as asexual for decades, genome analyses as well as population genomics studies and crossing experiments in the laboratory have led to the discovery of sexual propagation in many presumed asexual fungal species (Dyer and Kück 2017). Therefore, it is currently not known whether any truly asexual fungal lineages exist that have completely lost the ability to undergo sexual propagation. In the following sections, we will discuss current knowledge in the two largest fungal groups, the Ascomycota and Basidiomycota, and then briefly mention some recent results for the Glomeromycotina, a group of plant symbionts that have been discussed as a long-term asexual group of fungi. We will finish the review with some thoughts on the continuum of sexual versus asexual propagation in fungi.

### 7.5.1 *Modes of Reproduction in Ascomycota*

Ascomycota is named after their sexual sporangia (asci, singular ascus), and sexual propagation has been studied in great detail at the molecular level in a number of ascomycete model organisms (Bennett and Turgeon 2016; Zickler and Espagne 2016; Pöggeler et al. 2018). Especially the genes required for mating and meiosis

are well known in ascomycetes and can be used as molecular markers for the presence of cryptic sexual development in species where sexual propagation has not been observed yet. However, it has to be noted that meiotic genes may have functions outside of sexual reproduction, e.g., in stress-related ploidy changes as was recently shown in the human pathogenic fungus *Cryptococcus neoformans* (Zhao et al. 2020). Therefore, crossing experiments or population genetic studies are important to study actual sexual reproduction as described below. Mating in fungi is genetically regulated by so-called mating type (*MAT*) loci that contain at least one *MAT* gene (Kües et al. 2011; Bennett and Turgeon 2016). In ascomycetes, *MAT* genes often encode transcription factors that regulate downstream genes required for sexual reproduction. In self-sterile (heterothallic) ascomycete species, successful mating is only possible between partners with compatible *MAT* loci, whereas self-fertile (homothallic) species often encode compatible *MAT* genes within one genome (Heitman 2015; Pöggeler et al. 2018).

In the last century, fungal species for which no sexual stage was known from nature or laboratory observations were designated as “deuteromycetes” or “imperfect fungi,” and it was assumed that such species had lost the capacity to undergo sexual reproduction. Within the ascomycetes, this applied to up to 40% of surveyed taxa (Dyer and Kück 2017). However, population studies starting in the 1990s indicated that cryptic sex can exist in such species. The first study to show this analyzed polymorphic genetic markers in clinical isolates of *Coccidioides immitis*, the causal agent of the valley fever. Marker distribution in isolates was consistent with genetic recombination as opposed to clonal propagation of this fungus (Burt et al. 1996). Another line of evidence came after the first genomes of supposedly asexual species were sequenced in the early 2000s, and *MAT* genes as well as meiosis-specific genes were found to be present and to not have accumulated mutations (Pöggeler 2002; Galagan et al. 2005). A major breakthrough was achieved when it was shown that natural isolates of the supposedly asexual species *Aspergillus fumigatus* can undergo sexual development in the laboratory (O’Gorman et al. 2009). Since then, sexual reproduction under laboratory conditions was demonstrated for a number of supposedly asexual ascomycetes, and it is currently not clear if any truly asexual lineages can exist in the long term.

### 7.5.2 Modes of Reproduction in Basidiomycota

Similar to the Ascomycota, the Basidiomycota are named after their meiosporangium, the basidium, which in contrast to the ascus bears its spores externally instead of internal spore development. However, the life cycle is very similar with a sexual phase bearing meiotic spores and an asexual phase giving rise to millions of mitotic conidia in many lineages. As in Ascomycota, sexual structures are often unknown or overlooked, as they can be reduced to a few cells only being microscopically visible (Sampaio 2004; Oberwinkler 2017). Several lineages have managed to link developmental stages like parasitism or vector-based dispersal to the alternating

life cycle (Morrow and Fraser 2009). Sexual compatibility is usually mediated by two mating loci, one of which is coding for a pheromone/pheromone receptor system controlling syngamy, while the second is coding for homeodomain (HD) transcription factors relevant for maintenance of the dikaryon, regular cell divisions, and filamentous growth (Raudaskoski and Kothe 2010). The separation of the two mating loci on two chromosomes leads to a tetrapolar mating system in most Basidiomycota, with multiple alleles of the various genes in several lineages (Kües et al. 2011).

As the haploid phase of the life cycle is often characterized by a saprobic, yeast-like stage, most Basidiomycota from early diverging lineages are isolated as haploid cultures from nature and their sexual structures are unknown or at least not observed in culture. Genera like *Pseudozyma*, *Rhodospodium*, *Tilletiopsis*, or *Cryptococcus* were used to describe these so-called asexual species. Phylogenetic studies revealed that these genera are polyphyletic and mixed with sexual species suggesting overlooked sexual stages in some lineages (Begerow et al. 2000). However, several lineages like the genera *Malassezia*, *Moniliella*, *Tilletiopsis washingtonensis* s.l. seem to be completely asexual (without signs of sexual stages), although the mating genes seem to be present as in the case of *Malassezia* (Wang et al. 2015; Saunders et al. 2012). Many studies focused on mating under laboratory conditions to identify sexual structures. These studies could identify several mechanisms to maintain a sexual life cycle even without a compatible mating partner (Lin and Heitmann 2007, David-Palma et al. 2016) and such pseudo-sexual strategies might be common among Basidiomycota in several lineages (Coehlo et al. 2017). Functions of pheromones and pheromone receptors might be thus very diverse including functions not involved in mating and reproduction. Recently, it was shown that non-mating-type-specific receptors are common in Agaricomycetes (Kües et al. 2011), and therefore, functions of predicted pheromone receptors in potentially asexual lineages need to be elucidated to allow conclusive remarks on the presence or absence of sexual stages. At present, it is not yet clear if obligate asexuals exist among the Basidiomycota.

### 7.5.3 *Glomeromycotina: Ancient Asexuals or Cryptic Sex?*

A case in point about the difficulty of identifying truly asexual fungal species might be the Glomeromycotina. They belong to the Mucoromycota, a sister group to ascomycetes and basidiomycetes (Spatafora et al. 2017). The Glomeromycotina are mostly obligate plant symbionts that form the widespread arbuscular mycorrhiza with the roots of land plants (Lanfranco et al. 2016). Despite their environmental ubiquity, cultivation of Glomeromycotina in the laboratory is difficult due to their metabolic dependence on the host plant, and no sexual stages have been observed in nature, probably because their life is spent completely underground and thus they are difficult to observe in their natural environment. Glomeromycotina were considered as ancient asexuals; however, targeted searches for meiotic genes as well as genomic studies confirmed the presence of meiotic genes and putative *MAT* genes in Glomeromycotina genomes, making it likely that a sexual cycle exists in these

species (Halary et al. 2011; Tisserant et al. 2013; Lin et al. 2014; Ropars et al. 2016). Therefore, based on the available data, the Glomeromycotina cannot be described as ancient asexuals.

#### 7.5.4 Other Reproductive Strategies Influencing Genome Evolution

Given recent genomic insights in the distribution of sex-related genes and sexual propagation in fungi, it has been suggested that many fungal species might be considered not as completely sexual or asexual, but rather as consisting of isolates on a continuum of sexual reproduction ranging from fully fertile to asexual (Dyer and Kück 2017). This raises the question under what conditions a sexual or an asexual lifestyle might be advantageous specifically for a fungal species or isolate. One possible factor might be the degree of ploidy. Even though some fungi are diploids, the majority of ascomycetes and basidiomycetes harbor haploid nuclei. However, in filamentous fungi, the mycelia are usually coenocytic with two or more nuclei sharing a common cytoplasm (Maheshwari 2005). Furthermore, fungi can undergo vegetative hyphal fusions between different individuals of the same species leading to exchange of genetically different nuclei (Daskalov et al. 2017). Thus, deleterious mutations in one nucleus might be masked by functional copies in other nuclei, making the mycelia functionally similar to the cells of heterozygous diploid organisms (Fig. 7.1). However, especially hyphal fusion and subsequent nuclear exchange come with the risk of spreading, for example, infectious agents or transposons. Therefore, many fungi have evolved heterokaryon incompatibility systems that allow vegetative hyphal fusion only between compatible partners. Calculations based on allele frequencies for different incompatibility systems in the model fungus *Neurospora crassa* suggest that the likelihood for compatible interactions between germinating vegetative spores is rather low (Gonçalves et al. 2019). Thus, it is possible that at least in *N. crassa* propagation is biased toward sexual propagation (during which the vegetative incompatibility systems are turned off), because sexual propagation is limited to dedicated partitions of the mycelium, thereby preventing spreading of infectious agents to the rest of the mycelium.

Another point to be considered with respect to the advantages of sexual or asexual reproduction is the spreading of transposable elements (TEs), which in fungi could in principle occur during sexual propagation or during the above-mentioned vegetative hyphal fusion. It is interesting to note, though, that many sequenced fungal genomes have a low TE content compared to other eukaryotic genomes (Castanera et al. 2016; Spatafora et al. 2017; Stajich 2017). Studies in several fungal model organisms have revealed that at least five different genome defense systems evolved within the fungi that protect organisms from the spread of TEs and other repeats (Gladyshev 2017). In *N. crassa* alone, three genome defense mechanisms are known, one of which is active during vegetative growth, a second in the dikaryotic phase directly prior

to karyogamy, and the third during meiosis (Shiu et al. 2001; Gladyshev 2017). Thus, it appears that at least *N. crassa* has every contingency covered and is genetically prepared to counter transposon spread during sexual and asexual propagation. Genome defense in other fungi is less well studied, but it seems likely that genome defense mechanisms against transposable elements are present in some form in other species as well.

Population studies were performed for a few species only and therefore data on the relevance of recombination in fungi are broadly lacking. However, homothallism has been discussed as a common strategy to perform selfing and maintain at least parts of sexual recombination. For example, *Cryptococcus neoformans* is known to perform a unisexual or pseudo-sexual life cycle in addition to a classical sexual cycle (Lin et al. 2005; Ni et al. 2013). Thus, fungi display a huge variety of mixed forms between truly sexual and asexual species highlighting their great potential to adapt to diverse needs of reproduction. Obviously, facultative asexuality is here predominant as well as in other eukaryotes. A black-and-white system of sex/asex does not exist.

## 7.6 Asexual Genome Evolution and Epigenomics in Plants

### 7.6.1 Asexual Reproduction in Plants

Asexual reproduction occurs in land plants mostly in ferns, in a form called apogamy (Grusz 2016), and in flowering plants as apomixis, the asexual reproduction via seeds (Asker and Jerling 1992; Mogie 1992, see Box 1 for terminology). Land plants have a life cycle of alternating diplontic sporophytes (producing meiospores) and a haplontic gametophyte (producing gametes). Asexual reproduction keeps this life cycle but avoids meiosis–mixis cycles in many different ways. We will focus here on flowering plants. Apomixis is in angiosperms scattered across the phylogeny and occurs in about 2% of genera, with many different developmental pathways (Hojsgaard et al. 2014; Fig. 7.1). Studies on asexual genome evolution are scarce due to practical difficulties: first, plant genomes are complex and can vary dramatically in size (Michael 2014). Angiosperms have undergone ancient and recent genome duplications, resulting in gene duplications and diversification of gene functions (Jiao et al. 2011; Leebens-Mack et al. 2019). Second, asexuality does not occur in model organisms like *Arabidopsis* or in major crops plants. For this reason, genomic resources are also scarce. The only completely sequenced reference genomes for gametophytic apomixis is published from *Boechea*, a relative of *Arabidopsis* (Kantama et al. 2007; Kliver et al. 2018), and for sporophytic apomixis in *Citrus* (Wang et al. 2017). (Gametophytic and sporophytic apomixis are characterized by embryo development either from cells in the megagametophyte or directly from somatic cells in the sporophyte, respectively). Third, most research has focused so far on understanding genetic control mechanisms of apomixis rather than on evolutionary questions, with the major aim to introduce apomixis into crops (Ozias-Akins and Conner 2019).

Apomixis is heritable (Ozias-Akins and van Dijk 2007), but regulatory mechanisms turned out to be unexpectedly complex and rely mostly on differential expression of many genes that regulate the sexual pathway (Koltunow and Grossniklaus 2003; Sharbel et al. 2010; Hand and Koltunow 2014; Schmidt et al. 2014). Apomixis is usually facultative and occurs mostly in polyploids or in diploid hybrids. Recent studies on natural apomicts, however, suggest that apomixis emerges already at the diploid level and is then directly and indirectly established by polyploidy (Hojsgaard and Hörandl 2019). Facultative sexuality can also involve selfing (fertilization of egg cells with pollen from the same plant) as most apomicts are self-compatible (Hörandl 2010). Selfing is an otherwise common sexual pathway in plants (Schemske and Lande 1985), resulting in an increase of homozygosity and loss of genotypic diversity in the offspring. However, little is known about frequencies and effects of selfing in otherwise facultative apomictic lineages.

### 7.6.2 Case Studies on the Possible Effects of Muller's Ratchet in Plants

The first comparative study on asexual genome evolution used transcriptomes of flowering buds in the *Ranunculus auricomus* complex (Pellino et al. 2013). This system comprised obligately diploid sexual and facultative apomictic hexaploids. Transcriptome analysis by using dN/dS ratios revealed that both sexual and asexual genomes are under purifying selection without signs of genome-wide accumulation of deleterious mutations as evolutionary theory would predict (see above). The outlier genes with elevated non-synonymous to synonymous (dN/dS) ratios in the sexual/asexual comparisons belonged to genes involved in sporogenesis and gametogenesis, and hence may relate to functional aspects of apomixis rather than to mutation accumulation. However, the lack of a related reference genome hampered a comprehensive gene annotation. Nevertheless, signatures of allelic sequence divergence were detected in the hexaploid apomictic genomes, probably due to hybrid origin. The same system was studied using a mathematical model, incorporating empirical data on the degree of facultative recombination and different selection scenarios (Hodac et al. 2019). Results confirmed the hypothesis that even a low degree of facultative sexuality in these hexaploid apomictic lineages was sufficient to counteract Muller's ratchet. Purifying selection might be specifically efficient in the meiotically formed, haplontic gametophytes, a stage in which many genes are expressed, and hence deleterious mutations can be efficiently eliminated.

A similar study was performed on transcriptomes on four sexual/asexual species pairs of the genus *Oenothera* (Hollister et al. 2015). In this genus, asexual reproduction occurs in the quite unusual form of permanent translocation heterozygosity, i.e., a reciprocal translocation of chromosomes that results in a ring formation at meiosis such that chromosomes pair only at their tips. This ring form of meiosis results in complete suppression of meiotic recombination and segregation. Seeds are

usually formed via selfing; i.e., the parental genotype is maintained. Hollister et al. (2015) found indeed elevated levels of heterozygosity and increased accumulation of non-synonymous mutations in the asexual lineages compared to sexual species. This system supports the hypothesis that obligate asexuality results in mutation accumulation. However, also rare (facultative) outcrossing has been found in *O. biennis* (Maron et al. 2018), which might counteract Muller's ratchet.

A recent study using genome sequences on sexual/apomictic, diploid species pairs of *Boechera* revealed high levels of heterozygosity in apomicts, mostly due to hybrid origin (Lovell et al. 2017). Analysis of mutation accumulation was performed at different types of different genomic sites: (1) conserved non-coding sites, (2) conserved coding sites, (3) sites, where any mutation causes an amino acid substitution, and (4) sites where any mutation is synonymous. Mutation accumulation in asexuals was found to be significantly higher in categories (1) and (3), but not in (2), indicating that purifying selection is still present, but more relaxed in phylogenetically derived sites. Mutation accumulation was found to be independent from hybrid origin, although it is difficult to entangle contemporary mutations from the ancestral ones in the conspecific hybrid. The authors did not consider effects of facultative apomixis, although variable proportions of sexual/asexual seeds occurred in 11 of their 13 apomictic samples (Lovell et al. 2017). Facultative apomixis occurs also in other taxa of *Boechera* (Aliyu et al. 2010). Specific studies on evolution of RNA helicases in sexual and apomictic *Boechera* revealed that mutation accumulation is further depending on gene function (Kiefer et al. 2020).

Taken together, the presence of Muller's ratchet was overall confirmed in plants, but a little bit of sex seems to be sufficient to counteract the accumulation of deleterious mutations. The predominance of facultative sexuality and lack of ancient asexuals in plants fit to this scenario. The degree of facultative sexuality, however, is highly flexible in plants and can be influenced positively by environmental stress conditions (Klatt et al. 2016, 2018; Ulum et al. 2020). Such a stress response of the reproductive mode is ploidy-dependent (Ulum et al. 2020). The possible combination of apomixis to selfing adds another level of complexity to understand mutation dynamics in asexual plant lineages. These dynamics, however, have not yet been investigated.

### 7.6.3 Studies on the Epigenome and Transposable Elements

Plant mode of reproduction can have consequences for the proper functioning of the epigenetic mechanisms that suppress TE activity. Epigenetic mechanisms, and specifically DNA methylation, silence TEs, can modulate gene expression. In plants, DNA (cytosine) methylation occurs in all sequence contexts (CG, CHG, and CHH, where H = C, T or A). The enzymatic pathways for depositing and maintaining methylation marks differ between contexts, as do their functions and dynamics. Broadly speaking, DNA methylation in plant gene bodies often occurs in the CG

context, which shows strong transgenerational stability but unclear functional relation to gene expression (Wendte et al. 2019). TEs can be densely methylated in all sequence contexts, which is under active control by a small RNA-guided DNA methylation mechanism (RdDM, RNA-directed DNA methylation) and which is associated with transcriptional silencing (Slotkin and Martienssen 2007; Matzke et al. 2015). The pathways that lead to DNA methylation silencing of TEs involve small RNAs that are used to guide methylation to specific TE loci (Matzke et al. 2015).

Because large portions of plant genomes can consist of TEs (for instance, up to 85% in the maize genome; Schnable et al. 2009), the epigenetic mechanisms that protect the genome from their uncontrolled transposition have potentially large consequences for genome evolution. In recent years, asexual plants have become popular model systems to study the causes and evolutionary consequences of DNA methylation variation (Richards et al. 2017), because (1) epigenetic effects are more easily detected in uniform genomic backgrounds, and (2) epigenetic variation is hypothesized to be of comparably high relevance for adaptation in asexuals that show little DNA sequence variation (Verhoeven and Preite 2014).

A relevant proximate question related to the impact of epigenetic mechanisms on asexual genome evolution is: How does the absence of meiosis in asexuals affect the stability and genomic patterns of epigenetic variation, and what consequences does this have for genome evolution? While detailed analysis in asexual plants is limited by the availability of high-quality reference genomes (Richards et al. 2017), relevant insights come from understanding the epigenetic processes that take place during sexual plant reproduction. In comparison to mammals, which undergo extensive DNA methylation erasure during gametogenesis and early embryogenesis (Feng et al. 2010), plants experience relatively limited DNA methylation resetting between generations. DNA methylation in CG contexts in particular shows high transgenerational stability (Johannes et al. 2009). However, DNA methylation that is under control of the RdDM pathway shows interesting dynamics: in both male and female germlines, cells that accompany the germ cells, but not the germ cells themselves, undergo active demethylation (Ibarra et al. 2012); the endosperm shows reduced DNA methylation levels, and the developing embryo shows a gradual increase in non-CG methylation (Bouyer et al. 2017).

In pollen, the loss of DNA methylation in the vegetative cell (which does not contribute genetic information to the next generation) releases TE activity, which results in TE-derived expressed transcripts that are subsequently degraded into small RNAs. It has been shown that these small RNAs are transported to the sperm cells (which do contribute to the next generation) where they can be used by the RdDM machinery to target corresponding TE sequences for silencing (Martinez et al. 2016). A similar process seems to occur in female plant germ lines (Ibarra et al. 2012). It is believed that this mechanism functions to reinforce TE silencing in the germ cells and the resulting embryo (Slotkin et al. 2009).

Asexual reproduction that does not involve the above germline epigenetic processes may therefore result in less efficient epigenetic silencing of TEs. For instance, lack of sex-reinforced TE silencing is thought to underpin an abnormal fruit phenotype (“mantled” fruit that produces less oil) that arose in oil palms under



tissue culture. This stable phenotypic variant is caused by loss of methylation in a LINE retrotransposon (Ong-Abdullah et al. 2015). Beyond variable silencing of TEs, we can speculate that compromised silencing results in increased transposition rates over longer evolutionary time scales, thereby contributing to mutational degeneration of asexual lineages.

## 7.7 Conclusion and Outlook

It has become clear that most of the classical predictions on the disadvantages of asexual reproduction need (re-)evaluation on a whole-genome basis. Moreover, for testing most hypothesis on the maintenance of sex, it is imperative to compare replicates of independently derived asexual lineages to closely related sexual species at both the population level and species level to disentangle true consequences of asexuality from confounding lineage-specific patterns. Such comparative studies are needed for a phylogenetically broad representation of the eukaryote tree of life (Burki et al. 2019).

The classical prediction that sex is imperative for effective purging of deleterious mutations is not universally met. It remains an open question whether large population sizes and/or effective repair mechanisms facilitate effective selection in ancient asexuals. Importantly, if strategies exist that avoid long-term detrimental effects of asexuality, research efforts should focus more on the immediate, short-term benefits of sex that require identification of eco-evolutionary dynamics. Facultative asexuality might occur much more often than expected in animals and fungi, which might alter the perception of costs and benefits of sex. More knowledge is required on the frequency of transitions to asexuality in natural populations as well as identifying its underlying molecular mechanisms and cytology.

The diversity of different developmental pathways and genomic features of asexual eukaryotes needs to be considered for empirical genome studies. The prevalence of mixed systems (such as facultative, cyclical, or intermittent sexuality also often combined to selfing) complicates predictive models. Why are not all eukaryotes capable of such mixed systems with a “little bit of sex,” which would preserve both variability and maintenance of favorable genotypes, both in the short and long term? Perhaps we should ask the question why and how sex has ever become obligate? Here, a better understanding of regulatory mechanisms and functions of meiosis–mixis cycles will be essential. Genomic studies beyond mutation screenings, targeting DNA repair and mutagenesis as well as epigenetic effects and TE dynamics, are needed to understand the actual advantages of obligate sexuality compared to facultative or obligate asexuality.

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