The Use of dN/dS Ratios to Investigate Types of Selection in Related Sexual and Asexual Lineages

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Abstract

With the increasing availability of whole-genome assemblies, interest in the genomic consequences of diverse reproductive modes has grown. Reduced efficiency of selection in asexually reproducing species is often cited as a major consequence and is frequently investigated using the ratio of nonsynonymous to synonymous substitutions (dN/dS). However, many studies do not give sufficient attention to the fundamental differences between nonsexual reproductive modes, which likely have substantial effects on the efficiency of selection. We reviewed studies that compared dN/dS ratios of sexual and asexual relatives across 20 taxa. Asexual reproductive modes were classified into two categories: Meiosis without interchromosomal mixis (automixis and premeiotic doubling), i.e. modified meiosis that restores ploidy without outcrossing, and apomixis, i.e. production of unreduced eggs that develop without meiosis and without interchromosomal or intrachromosomal mixis. We further included two modes of reproduction which are not sexual and not strictly asexual, but may be seen as such at first glance: Fissiparity/clonal growth, i.e. the formation of new modules/ramets by splitting of somatic tissue without meiosis or mixis, and selfing, i.e. meiosis followed by fertilization of the gametes by the same individual. Consistent with theory, fissiparous lineages generally exhibited elevated dN/dS ratios, whereas automicts and apomicts showed no consistent pattern, even among taxa with identical reproductive modes. We discuss limitations of commonly used phylogenetic analyses using maximum likelihood (PAML) branch and branch-site models, which can obscure subtle differences, and propose different avenues to assess detection of selection. We propose that the heterogeneous patterns in asexual lineages reflect lineage-specific consequences of asexuality, model limitations, and our incomplete understanding of cytological mechanisms underlying different nonsexual reproductive modes.

Key words: dN/dS ratios, sexuality, asexuality, clonal growth, selfing, codon evolution.

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Significance

An organism's reproductive mode fundamentally shapes its genomic landscape, yet the consequences of noncanonical reproductive modes remain poorly understood. Although reduced selective efficiency is often predicted in asexual species, no consistent trend has been observed. The efficiency of selection is commonly investigated using dN/dS ratios, but most studies conflate distinct forms of asexuality and their underlying cytological mechanisms, and this limits interpretation. Here, we review studies comparing sexual and asexual relatives across 20 metazoan and angiosperm taxa, categorizing lineages by reproductive mode. We highlight limitations of traditional phylogenetic analyses using maximum likelihood (PAML) dN/dS models and suggest alternative approaches from the Hypotheses Testing Using Phylogenies (HyPhy) suite for more tailored analyses. By clarifying lineage-specific traits and methodological constraints, our synthesis provides a roadmap for future studies examining evolutionary differences between sexual and asexual relatives.

Introduction

The reason for the dominance of sexual reproduction among eukaryotes is one of the major questions of evolutionary biology (Maynard Smith 1971; Bell 1982; Neiman et al. 2017). Asexually reproducing lineages (asexuals) should outperform bisexual relatives due to the 2-fold advantage of **parthenogenesis** with respect to nonproducing parental individuals. Further, all asexuals should benefit from the prevention of genome dilution and the maintenance of well-adapted gene combinations (Lehtonen et al. 2012). A wide range of theories have been proposed to explain the evolution of **sex**, but no consensus has been reached (West et al. 1999; Scheu and Drossel 2007; Hörandl 2009; Burke and Bonduriansky 2017; Brandeis 2017). One potential factor often discussed in these theories is the accumulation of deleterious mutations caused by the reduced efficiency of natural selection due to the lack of **outcrossing** (Muller 1964; Kondrashov 1988; van der Kooi and Schwander 2014); for the definition of terms in bold used in this review, see Glossary. Testing such hypotheses requires evolutionary replicates, i.e. either closely related sexual and asexual lineages, or taxa with multiple independent transitions to asexuality. Such replicates allow comparison of genomic parameters with little ecological and evolutionary divergence.

The most prominent genomic tool to infer reduced selective efficiency among evolutionary replicates is the ratio of nonsynonymous (dN) to synonymous (dS) nucleotide substitutions in protein-coding genes. Different codons may code for the same amino acid, due to the degenerated nature of the genetic code (Crick et al. 1961; Nirenberg and Leder 1964). A substitution that changes the amino acid is called nonsynonymous, while one that does not is called synonymous. **Purifying selection** is assumed to remove deleterious amino acid–changing mutations, while synonymous substitutions are largely neutral and reflect the baseline mutation rate (Nei and Gojobori 1986; Yang and Bielawski 2000; Kryazhimskiy and Plotkin 2008). However, selection can also act at the nucleotide level, for example through codon usage bias or regulatory constraints such as alternative splicing (Hershberg and Petrov 2008). Consequently, a **dN/dS ratio** below one is typically interpreted as evidence for purifying selection, whereas a ratio above one indicates **positive** or diversifying **selection** (Yang and Bielawski 2000; Kryazhimskiy and Plotkin 2008). A dN/dS ratio close to one is often taken as an indication of neutrality, but this interpretation can be misleading, as it may also reflect a mixture of sites under purifying and positive selection that average out (Kryazhimskiy and Plotkin 2008). It is important to note that most organisms operate close to a selective optimum, resulting in positive **adaptive selection** being relatively rare (Eyre-Walker and Keightley 2007; Hughes 2007). Moreover, negative mutations are by far more frequent than positive ones. The concept of **relaxed selection** is especially useful in this context, as it describes a reduction in the efficiency of purifying selection rather than a shift toward adaptive evolution (Lahti et al. 2009; Wertheim et al. 2014). A central factor influencing the strength of purifying selection is the effective population size (N_e; Kimura 1983). In large populations, deleterious mutations are efficiently removed, whereas in small populations they may drift to fixation. This effect is amplified in lineages experiencing frequent population bottlenecks where drift overcomes selection (Nei et al. 1975).

In diploid asexuals, the N_e is expected to be approximately halved relative to sexual populations of equal census size, due to the absence of recombination and the transmission of identical genomes across generations (Kimura 1983; Otto and Lenormand 2002; Charlesworth 2009). A reduced N_e decreases the efficacy of purifying selection, allowing mildly deleterious mutations to accumulate and thereby increasing dN/dS ratios (Ohta 1973; Kimura 1983; Eyre-Walker and Keightley 2007). In addition, depending on the mode of asexual reproduction (see below), heterozygosity may be reduced (Suomalainen et al. 1987; Blanc et al. 2025). Reduced heterozygosity can expose recessive deleterious alleles to selection, potentially enhancing purifying selection and counteracting increases in dN/dS (Charlesworth and Charlesworth 1999; Glémin 2003; Glémin et al. 2019). However, the efficiency of such purging critically depends on N_e, as exposed deleterious alleles are more effectively removed in large populations than in small ones (Kimura 1983; Gravel 2016). Furthermore, asexuality results in genome-wide linkage, which intensifies genetic hitchhiking and background selection, further reducing N_e and elevating dN/dS ratios (Hill and Robertson 1966; Charlesworth et al. 1993). Importantly, some asexual lineages are demographically successful and attain large population sizes, and may therefore maintain high N_e despite the absence of sex (e.g. oribatid mites; Heethoff et al. 2009; Neiman et al. 2014). Consequently, variation in dN/dS ratios among asexual taxa may reflect ecological and demographic factors as much as, or more than, reproductive mode per se.

Types of Asexuality and Their Consequences

Studies investigating differences in dN/dS ratios between sexual and asexual lineages typically use broad terms such as "asexuality" or "parthenogenesis". These broad terms ignore the differences of the various types of asexuality, and this may have contributed to the ambiguous results published in the past (Table 1). Here, we define sex as the alternation of meiosis, resulting directly or indirectly (e.g. via gametophytes) in reduced gametes, and the fusion of those gametes, whether they arise from the same or different individuals. During these processes, two types of recombination (or mixis) occur. First, intrachromosomal mixis during crossing-over events (meiotic recombination), and second, interchromosomal mixis during fertilization (gametic recombination due to outcrossing).

In this review, we distinguish two major categories of asexuality based on their cytological and genetic underpinnings (Fig. 1). Automixis and premeiotic doubling entail reproduction through modified meiosis, in which uniparental meiotic products fuse to restore diploidy; depending on the cytological modification, this can result in varying levels of heterozygosity loss (Suomalainen et al. 1987; Engelstädter 2017; Blanc et al. 2025). Apomixis involves the clonal development of an unreduced egg cell or somatic cell without fertilization and without meiosis, producing genetically identical offspring (Asker and Jerling 1992; Richards 1997; Simon et al. 2003).

Further, we investigate **fissiparity/clonal growth** as well as self-fertilization (**selfing**). Fissiparity (or clonal growth) is the splitting of somatic tissue into new modules or ramets, resulting in clonal propagation without meiosis or fertilization (Hughes 1989; Otto and Gerstein 2006). It may not be classified as asexual reproduction in the strict sense because it does not involve the development of a new organism from an unspecialized, single-cell stage. Thus, fissiparity is more accurately described as propagation or clonal growth rather than true "reproduction" (Mogie 1992). While this distinction is straightforward in eukaryotes with differentiated tissues (e.g. animals and land plants), it becomes difficult in protists, fungi, and algae, and most authors therefore include vegetative fission under asexuality (see overview in Hörandl et al. 2020). Here, however, we treat fissiparity as neither sexual nor asexual, because the division of a multicellular organism into two viable units represents growth and reorganization of existing tissues rather than the formation of a new organism. In this regard, it is comparable to developmental processes such as metamorphosis in holometabolous insects (Huxley 1852), where cellular death and proliferation reshape an existing individual rather than generate a genetically "new" one. Selfing refers to reproduction via self-fertilization in hermaphroditic species, which involves two different (male and female) meiotic divisions and fertilization of the gametes by the same individual (Jarne and Charlesworth 1993). This is more akin to extreme inbreeding (i.e. sex) and should be viewed as a separate category from asexuality (Brandeis 2017). In botany, selfing is common and usually regarded as a form of sexual reproduction (Barrett 2002). While these categories are not strictly asexual, we include them as they are often cited in papers analyzing dN/dS ratios between "true" asexuals and their relatives.

Here, we review studies that utilized dN/dS ratios to identify genomic differences between sexual and asexual embryophytes and metazoans, with particular attention to how different modes and origins of asexuality may affect the efficiency of selection. Traditionally, one would expect higher dN/dS ratios in asexuals compared to sexual relatives due to relaxed purifying selection on negative nonsynonymous mutations, which accumulate over time.

Automixis and Premeiotic Doubling

Automixis involves a number of meiotic modifications that allow unfertilized eggs to restore diploidy through the fusion of nuclei from the same meiotic event, eventually resulting in the production of nonclonal offspring (Suomalainen et al. 1987; Engelstädter 2017; Blanc et al. 2025). Blanc et al. (2025) categorized these modifications into three main types (though other specialized mechanisms and modifications may exist; Fig. 1):

Table 1 Studies investigating differences in dN/dS ratios between asexual species and sexual relatives sorted by mode of asexual reproduction

Mode of asexuality	Study	Taxon	…	dN/dS ratio	Age of nonsexual lineage	Reproductive mode(s) of sexual relatives
Automixis	Paland and Lynch (2006) and Tucker et al. (2013)^{X}	Metazoa	*Daphnia pulex* (H)	Higher in asexuals*	<1,000 years	Cyclical parthenogenesis
…	Brandt et al. (2017)	Metazoa	Oribatida	Higher in sexuals*	Up to 20 million years (Öztoprak et al. 2025)	Sex
…	Kočí et al. (2020)	Metazoa	*Cobitis taeina* (H)	No significant differences	Up to 300,000 years	Sex
…	Maldonado et al. (2022)	Metazoa	*Aspidoscelis* (H)	Higher in asexuals	~360,000 years (*A. tesselata*)	Sex
Apomixis	Lunt (2008)	Metazoa	*Meloidogyne* (H)	No significant differences	Recent (no estimate given)	Sex
…	Neiman et al. (2009)	Metazoa	*Potamopyrgus antipodarum*	Higher in asexuals	500,000 years for old, < 40,000 years for young asexuals	Sex
…	Pellino et al. (2013)	Angiosperms	*Ranunculus auricomus* (H)	No significant differences	~80,000 years	Sex
…	Hollister et al. (2015)	Angiosperms	*Oenothera* (H)	Higher in asexuals	12,014 to 506,477 years	Sex
…	Lovell et al. (2017) and Kiefer et al. (2020)	Angiosperms	*Boechera*	No significant differences	/	Sex
…	Henry et al. (2011) and Bast et al. (2018)	Metazoa	*Timema*	Higher in asexuals	0.2 to 2.0 million years (Schwander and Crespi 2009; Schwander et al. 2011)	Sex
…	Yan et al. (2022)	Metazoa	*Trichogramma* (H)	No significant differences	Recent (no estimate given)	Haplodiploidy
…	Yarbrough and Chandler (2024)	Metazoa	*Trichoniscus pusillus* (and relatives) (H)	Higher in sexuals	Not real evolutionary replicates	Sex
Fissiparity/clonal growth	Ament-Velásquez et al. (2016)	Metazoa	*Lineus* (H)	Higher in nonsexuals*	Recent (no estimate given)	Sex/Fissipariy
…	Kershenbaum et al. (2024)	Metazoa	*Schmidtea mediterranea*	Higher in nonsexuals*	/	Sex/Fissipariy
…	Wang et al. (2025)	Angiosperms	Bambusoideae (and relatives)	Higher in nonsexuals	/	/
Selfing	Wright et al. (2002)^{1} Payne and Alvarez-Ponce (2018)^{2}	Angiosperms	*Arabidopsis* (H)	^{1}No significant difference ^{2}Higher in selfers	0.1 to 1.0 million years	Sex
…	Haudry et al. (2008); Escobar et al. (2010)	Angiosperms	Triticeae (H)	No significant difference	/	Sex
…	Cutter et al. (2008)	Metazoa	*Caenorhabditis*	Higher in selfers*	/	Sex
…	Burgarella et al. (2015)	Metazoa	Gastropoda	Higher in selfers	/	Sex
…	Wang et al. (2020)	Angiosperms	*Primula* (H)	Higher in selfers	/	Sex

(H) = Asexual lineages of hybrid origin; * = Only some data/gene sets used produced significant results; X = *Daphnia pulex* classified as apomictic, but recent cytological findings suggest that they are automictic (Hiruta et al. 2010; Xu et al. 2011).

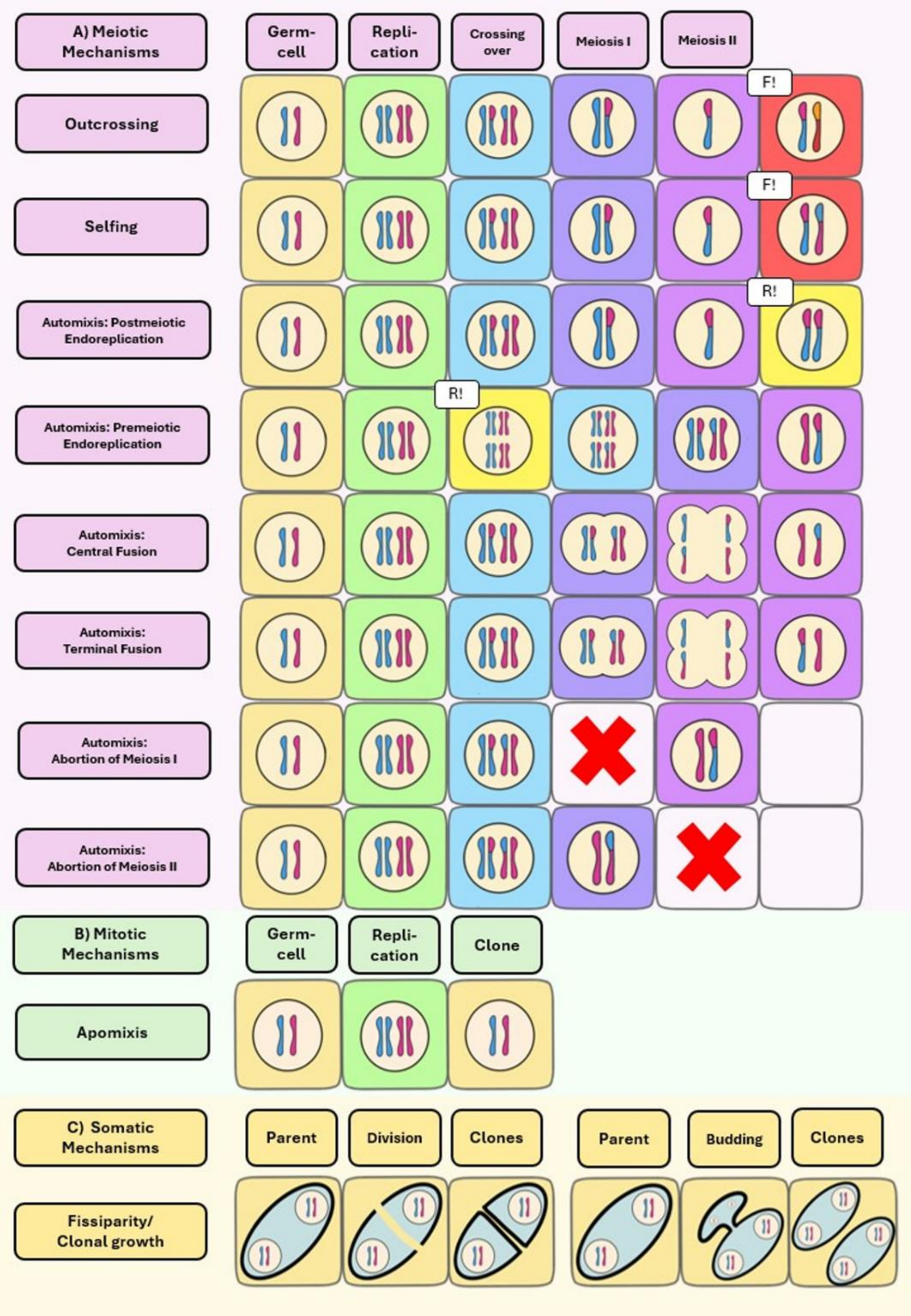

Fig. 1. Overview of the mechanisms underlying the reproductive modes reviewed in this study. The modes are organized according to overarching mechanisms: a) meiotic, b) mitotic via germ cells and c) somatic. The genome is depicted as diploid for simplicity. The singular steps are coded by color: Germ-cell = beige, replication = green, crossing-over = light blue, meiosis I = dark blue, meiosis II = purple. Further, fertilization is marked red and additional genome duplications are marked yellow. Note that this overview is simplistic and does not accurately depict the full diversity and nuances of eukaryotic reproduction. Further note that the depiction of apomixis does not comprehensively capture the complexity of asexual reproduction in plants. For a detailed figure, see additional material 1 (Hörandl 2024). F! = fertilization, either via outcrossing or selfing. R! = additional chromosome replication for the restoration of ploidy.

(i) Two-step meiosis with chromosome endoreplication involves duplication of chromosomes either before or after meiosis to maintain ploidy. When endoreplication occurs before meiosis (premeiotic), recombination can occur between identical sister chromatids or, in some taxa, between divergent homologs. The extent of recombination between nonsister chromatids varies among lineages. This generally preserves heterozygosity, whereas post-meiotic endoreplication can result in partial loss of heterozygosity due to pairing between previously divergent chromatids. (ii) Two-step meiosis with fusion of meiotic products (automixis), where the oocyte fuses with a polar body to restore diploidy. Fusion with the first polar body (central fusion) generally preserves heterozygosity, whereas fusion with the second (terminal fusion) rapidly increases homozygosity (Engelstädter 2017; Blanc et al. 2025). (iii) Abortion of one meiotic division, in which skipping meiosis I prevents homologous segregation and preserves heterozygosity, while skipping meiosis II allows homologs to segregate but not sister chromatids, leading to increased homozygosity. Diplospory in plants is a form of apomixis that may retain a restitutional meiosis (either skipping meiosis I or II), making it analogous to automixis. Moreover, Blanc et al. (2025) highlighted inverted meiosis, where meiosis II occurs before meiosis I, and one of these steps is usually aborted. Depending on which division is aborted in automicts, zygosity outcomes resemble those of canonical meiosis: skipping meiosis I largely preserves heterozygosity, whereas skipping meiosis II increases homozygosity.

The type of automixis and the specific meiotic modification determine how much heterozygosity is retained in offspring. This in turn can influence the effectiveness of purifying selection, because higher homozygosity exposes recessive deleterious alleles while also reducing recombination, thereby shaping patterns of dN/dS ratios among lineages (Lynch et al. 1993; Neiman et al. 2014).

Only a few studies have investigated dN/dS ratios in automictic taxa (Table 1). Two notable examples are Maldonado et al. (2022), who studied the genus *Aspidoscelis* (Squamata), and Kočí et al. (2020), who examined the *Cobitis taenia* species complex (Actinopterygii). Both systems include sexual lineages and asexual hybrid polyploids that reproduce via premeiotic endoreplication. Maldonado et al. (2022) analyzed both mitochondrial and nuclear genes and reported significantly higher mitochondrial dN/dS ratios and evidence for relaxed selection in asexual *Aspidoscelis* lineages. By contrast, Kočí et al. (2020) found no evidence for relaxed selection in the mitochondrial genes of asexual *C. taenia*, which reproduces via premeiotic doubling and gynogenesis, a reproductive mode in which a male gamete triggers development but its genome is excluded from the zygote. Kočí et al. (2020) likewise detected no significant differences in dN/dS ratios between sexual and asexual lineages for nuclear genes.

At first glance, comparisons of mitochondrial (and/or plastid) genes between sexual and asexual lineages may appear inappropriate given their predominantly maternal inheritance. However, because organelle genomes are not recombining via meiosis and have a reduced effective population size relative to nuclear genomes, they are particularly susceptible to the accumulation of deleterious mutations through genetic drift and Hill–Robertson interference (Hill and Robertson 1966; Havird et al. 2015). Gene conversion in organelle genomes and cell-to-cell variability are efficient mechanisms to create genetic variance that counteracts mutation accumulation (e.g. Khaklova and Bock 2006, Mower et al. 2010, Edwards et al. 2021). In sexual lineages, nuclear-encoded genes for organelle function are recombined and can evolve rapidly counter-adaptations to compensate for organelle mutation accumulation (Havird et al. 2015). In asexual lineages, where nuclear recombination is also reduced or absent, mutation accumulation may be exacerbated, leading to an increased effect of organelle mutation load and potentially relaxed purifying selection (Havird et al. 2015).

Methodological differences may also contribute to the contrasting results between studies. Kočí et al. (2020) restricted their mitochondrial analyses to a method identifying shifts in the intensity of selection (RELAX, Wertheim et al. 2014), whereas Maldonado et al. (2022) additionally applied branch models. Finally, lineage-specific factors such as life-history traits, clone age, and effective population size may further explain the observed discrepancies (Jaron et al. 2021).

Oribatid mites (Acariformes, Oribatida) are automicts and classified as "ancient asexuals" (Judson and Normark 1996; Brandt et al. 2021; Öztoprak et al. 2025). These are lineages which have been reproducing via obligatory parthenogenesis for prolonged periods of time. Brandt et al. (2017) observed slightly but significantly higher dN/dS ratios in 3,545 nuclear orthologs of sexual oribatid mites; however, the dataset included only six species. A subsequent analysis with more species but fewer genes revealed no significant differences between sexual and asexual species (Brandt et al. 2017). Interestingly, they observed significantly lower dN/dS ratios in ten mitochondrial genes of sexual oribatid mites (Brandt et al. 2017). This apparent lack of strong selective differences may explain the long-term maintenance of asexuality in this taxon. Whether this is linked to the cytological mechanism of producing parthenogenetic offspring is unclear, as the details remain debated. Proposed mechanisms include (i) a two-step meiosis with terminal fusion (Heethoff et al. 2009), (ii) inverted meiosis with abortion of meiosis I and biased "Z segregation" of chromatids (Archetti 2021), or (iii) two-step meiosis with crossovers restricted to chromosome ends (Öztoprak et al. 2025). However, none of these scenarios is firmly supported by cytological evidence, and early descriptions remain fragmentary (Taberly 1958). This uncertainty makes it difficult to predict if and how automixis shapes heterozygosity and selection in oribatid mites.

While the *Daphnia pulex* complex (Cladocera) is traditionally assumed to be apomictic (Herbert and Crease 1980; Paland and Lynch 2006; Tucker et al. 2013), recent cytological studies observed an abortion of meiosis I followed by a regular meiosis II (Hiruta et al. 2010; Xu et al. 2011) as clarified by Blanc et al. (2025). Following this, we classified this species complex as automictic, with some lineages exhibiting cyclical parthenogenesis. Paland and Lynch (2006) found higher dN/dS ratios in the mitochondrial genes of the obligate automict *D. pulex* lineages than in the ones reproducing via cyclical parthenogenetic. Interestingly, however, they found no significant differences in dN/dS ratios in nuclear genes. Contrasting these findings, Tucker et al. (2013) reported higher dN/dS ratios in certain genes of obligate asexual *D. pulex* lineages compared to cyclical parthenogenetic lineages. However, they assumed that these gene-specific elevated dN/dS ratios were already present before the transition to obligate asexuality, which was rather recent (<1,000 years). These results indicate that (i) cytological reinvestigations of auto- and apomicts are of eminent importance, and (ii) certain genes, such as the ones related to asexuality and mixis, may deviate from genes with other functions.

Overall, the genomic effects of automixis and related cytological mechanisms remain elusive due to the diversity of cytological mechanisms, differences in life histories and the few studies existing. Further genome-wide analyses investigating the dN/dS ratios of automictic lineages using varying cytological mechanisms are necessary to allow more conclusive interpretations of the genetic and cytological characteristics of automixis.

Apomixis

Apomictic asexuality usually entails the development of an unfertilized egg cell. Notably, it lacks both mixis and meiosis and therefore produces direct clones of the mother (Fig. 1). Whether this strict definition applies to all taxa discussed here, however, remains uncertain, as Jaron et al. (2022) and Blanc et al. (2025) demonstrated that many classical examples of apomixis in metazoans actually undergo some form of meiosis and therefore are automictic. In flowering plants, some forms of apomixis undergo a restitutional meiosis, resulting in unreduced gametophytes producing unreduced egg cells, which can develop without fertilization (diplospory). Other forms of apomixis involve the formation of unreduced gametophytes and egg cells out of unreduced somatic cells of the ovule (**apospory**), or direct development of embryos out of unreduced diploid somatic cells of the ovule (**adventitious embryony**). Most apomictic flowering plants can produce both sexual and asexual seeds in the same generation, i.e. are facultative apomicts (see review of Hörandl 2024).

For genome evolution, it is important to which extent apomixis is obligate. Theory and mathematical models predict that low levels of recombination, e.g. via cyclical or facultative apomixis, are sufficient to counteract mutation accumulation (Green and Noakes 1995). One well-studied case of obligate asexuality is the angiosperm genus *Oenothera*. In *Oenothera*, permanent translocation heterozygosity reduces recombination during fertilization to near zero, resulting in two independently evolving haploid genomes (Cleland 1974). Combined with selfing, this makes *Oenothera* reproduction functionally apomictic (Wang et al. 2020). Hollister et al. (2015) investigated selection in sexual and asexual *Oenothera* lineages and found increased dN/dS ratios in the asexuals. In the genus *Boechera*, both obligate and facultative asexuals exist, and apomictic species evolved multiple times via hybridization (Lovell et al. 2017). Analyzing genome evolution, the authors found site-specific patterns, but asexual genotypes harbored more mutations in highly-conserved sites than related sexuals, with effects being least pronounced in coding sites. The dN/dS analyses by Kiefer et al. (2020) on sexual and asexual *Boechera* species, focusing on RNA helicases, also revealed higher variation between different genes rather than between modes of reproduction. Similar results were reported in the stick insect genus *Timema* (Henry et al. 2011; Bast et al. 2018). *Timema* is particularly interesting because apomixis evolved multiple times independently without evidence for hybridization. Bast et al. (2018) noted that, although dN/dS ratios were higher in asexuals, these lineages did not show signs of reduced fitness, which may reflect their relatively recent origin or other lineage-specific factors. High dN/dS ratios were also observed in the mitochondrial genome of the freshwater snail *Potamopyrgus antipodarum* (Neiman et al. 2009). In this system, mixed populations of obligate sexual and asexual individuals coexist, and infection by the sterilizing trematode *Microphallus* sp. is thought to influence this balance rather than directly inducing parthenogenesis (Lively 1987; Vergara et al. 2014).

Other studies, however, found no significant differences between sexuals and apomicts. Lunt (2008) reported no differences in dN/dS ratios between sexual and asexual root-knot nematodes of the genus *Meloidogyne*. Once thought to be an "ancient asexual scandal", it is now understood that *Meloidogyne* has a more recent hybrid origin of asexuality (Lunt 2008). Likewise, Pellino et al. (2013) found no differences in the facultative apomictic angiosperm *Ranunculus auricomus* complex, where asexual lineages are relatively young (~80,000 years). Interestingly, their genome-wide analysis identified outlier genes with highly divergent dN/dS ratios; several of these were involved in meiosis and gametogenesis, suggesting a functional association with apomixis and possible positive selection. Elevated dN/dS ratios for functional apomixis candidate genes only could be later confirmed in apomictic F2 hybrids of *R. auricomus* lineages, ruling out long-term mutation accumulation (Ptzold et al. 2022). Offspring analysis of the plants used in Pellino et al. (2013), modeling the effect of facultative sex on mutation accumulation, found that as little as 6% recombinants per generation are sufficient to halt Muller's ratchet (Hodac et al. 2019), confirming that "a little bit of sex" may be as good as obligate sex (Hojsgaard and Hörandl 2015). For flowering plants, strong selection acting on reduced versus unreduced gametophytes, in which the majority of genes are expressed, may explain this pattern (Hojsgaard and Hörandl 2015). No differences were also detected in the mitogenomes of two apomictic *Trichogramma* species, one of hybrid origin, the other induced by *Wolbachia* (Yan et al. 2022). In a broader comparative study, Brandt et al. (2019) also found no consistent trend in apomictic hexapods and their sexual relatives.

Finally, there is one case in which sexual lineages exhibit slightly higher dN/dS ratios than asexuals. Yarbrough and Chandler (2024) reported this pattern in the sexual terrestrial isopod *Hyloniscus riparius* compared to its relative *Trichonisus pusillus*, where asexual lineages consist of diploid and triploid hybrids coexisting with sexual lineages. It is important to note, however, that this study lacks true evolutionary replicates as sexual individuals of *T. pusillus* were not included, introducing potential bias. Nevertheless, this pattern resembles that of automictic oribatid mites (Brandt et al. 2017), and both groups share ecological similarities as soil-dwelling detritivores.

Overall, studies on apomixis show a convoluted picture with no consistent trend in higher dN/dS ratios in apomicts than in sexual species/lineages. Lineage-specific traits, including age, origin and ecological context, appear to play a more important role in shaping patterns of genome degeneration in apomicts than apomixis itself (Jaron et al. 2021). Moreover, different gene-specific evolutionary patterns can strongly influence dN/dS ratios that were calculated over whole genomes or transcriptomes.

Fissiparity/Clonal Growth

Fissiparity and clonal growth involve the production of genetically identical offspring through fragmentation or vegetative propagation (Huxley 1852; Coe 1929; Bierne 1970; Fig. 1). In animals, fissiparous reproduction relies on regeneration of body parts, whereas in plants, clonal propagation occurs through rhizomes, stolons or other vegetative organs (Wang et al. 2025). From a broader perspective, fissiparity and clonal growth may be perceived as a special case of somatic growth in which modules split from the parental soma (Hughes 1989). Theory predicts that lineages reproducing exclusively through fissiparity or clonal growth should accumulate deleterious mutations more rapidly than sexual relatives. The absence of a single-cell stage during development, and of recombination and segregation, reduces the efficacy of purifying selection, potentially leading to increased dN/dS ratios (Muller 1964; Felsenstein 1974; Lynch et al. 1993; Grosberg and Strathmann 2007, Neiman et al. 2014). Within the nemertean genus *Lineus*, two species (*L. sanguineus* and *L. pseudolacteus*) can reproduce via fissiparity, owing to their ability to regenerate both anterior and posterior ends (Coe 1929). Both species usually lack adult gonads and are therefore suspected to reproduce primarily through asexual fragmentation (Bierne 1970; Ament-Velásquez et al. 2016). This genus also provides evolutionary replicates, as the sexual species *L. lacteus* and *L. longissimus* are closely related to the fissiparous species (Bierne et al. 1993; Ament-Velásquez et al. 2016). Population genomic analyses by Ament-Velásquez et al. (2016) revealed that *L. sanguineus* shows genomic patterns more similar to sexual species than to obligate asexuals. The authors attribute this to rare sexual events in individuals that develop fully functional female gonads. By contrast, *L. pseudolacteus* exhibits the expected genomic signatures of asexuality, including elevated dN/dS ratios and increased heterozygosity. Whether this reflects prolonged periods of obligate asexuality, however, is unknown, as this species appears to be a triploid hybrid.

Similar patterns are observed in other fissiparous metazoans. For instance, Kershenbaum et al. (2024) studied dN/dS ratio differences in fissiparous and sexual lineages of the planarian *Schmidtea mediterranea*. Like in the asexual *Lineus*, the fissiparous ability in these lineages is based on physical regeneration. The authors analyzed three sets of genes: (i) all protein-coding sequences, (ii) highly conserved single-copy BUSCOs, and (iii) sequences under significant purifying selection. They found that gene sets 1 and 3 had significantly higher dN/dS ratios in asexual lineages, whereas gene set 2 exhibited no significant differences between sexual and asexual lineages. The authors suggested that some unknown mechanism mitigates the loss in selective efficiency, resulting in comparatively modest differences between sexual and asexual strains.

In plants, similar trends occur in taxa with clonal reproduction. Wang et al. (2025) compared two sister taxa (subfamilies) within Poaceae, Pooideae, and Bambusoideae. These groups differ strongly in life-history traits and reproduction. Pooideae are mostly herbaceous, short-lived, and predominantly sexual, whereas Bambusoideae are long-lived woody plants that rarely reproduce sexually and rely on clonal growth. Analyses revealed that asexual Bambusoideae have significantly higher dN/dS ratios and more genes under relaxed selection than sexual Pooideae.

Taken together, studies of fissiparity in animals and clonal growth in plants support the hypothesis that purifying selection is, at least to some extent, reduced in these lineages, although the magnitude of this effect can vary depending on life history, regenerative capacity and rare sexual events.

Selfing

Generally, plants engage in outcrossing to counteract the increase in homozygosity, genetic drift and genetic linkage (Burgarella and Glemin 2017); however, obligate selfing may be favored by pollen limitation (Baker 1955, Barrett 2002). While the increase in homozygosity may enable selfing populations to purge deleterious and maintain beneficial recessive alleles (Glemin 2021), it may also lead to inbreeding depression, i.e. reduced offspring fitness (Lande and Schemske 1985). While in selfing plants, recombination is still present, effective recombination is heavily reduced as it mostly affects homozygous sites (Glemin et al. 2019). Most importantly, the effective population size is assumed to be heavily reduced in selfing species due to strong extinction/recolonization dynamics, genetic bottlenecks and other factors (Wright et al. 2013). The selective purging of recessive deleterious mutations is more frequently observed in annual selfing plants (Byers and Waller 1999).

Considering the high proportion and common transitions to high or obligate selfing in angiosperms (Igic and Kohn 2006; Landis et al. 2018), only a few studies have investigated the presumed reduction in selective efficiency, and even fewer metazoan hermaphrodites have been analyzed (Table 1). Haudry et al. (2008) and Escobar et al. (2010) investigated potential differences in dN/dS ratios among selfing and outcrossing Triticeae species (Poaceae, Angiospermae). All the species included were diploid. Neither study confirmed the hypothesis that selfers have lower dN/dS ratios due to reduced selective efficiency. However, it is important to note that both studies only included a small number of genes, i.e. 52 in Haudry et al. (2008) and 23 in Escobar et al. (2010). However, the relatively recent transition to obligate selfing in Triticeae species (Haudry et al. 2008; Escobar et al. 2010) may not have allowed to identify significant differences. Similarly, comparing dN/dS ratios of the predominant selfer *Arabidopsis thaliana* and the self-incompatible *A. lyrata*, Wright et al. (2002) found no significant differences; however, they also only used a small number of genes (23). Payne and Ponce (2018) reanalyzed the *Arabidopsis* genome using whole-genome data and found significantly higher dN/dS ratios in selfing lineages. Similarly, Wang et al. (2020) found higher dN/dS ratios in homostylous, highly selfing diploid *Primula* species analyzing 445 nuclear and 72 plastid genes. One of the two metazoans investigated for differences in dN/dS ratios is the nematode genus *Caenorhabditis* (Cutter et al. 2008). While the authors found no significant differences with a limited dataset, the highly selfing species *Caenorhabditis briggsae* (but not *Caenorhabditis elegans*) had higher dN/dS ratios when using a larger dataset. The second metazoan is the selfing freshwater gastropod *Galba truncatula*, which Burgarella et al. (2015) compared to its outcrossing relatives of the genus *Physa*. Using large-scale genomic data, they found slightly, but significantly increased dN/dS ratios in the outcrossing *G. truncatula*.

Overall, consistent with theoretical expectations, there is a trend of selfers having higher dN/dS ratios than closely related sexual species, indicating less efficient purifying selection, which may be detected if a sufficient number of genes are used for the analysis. However, only a few selfing lineages have been thoroughly investigated using whole-genome data. Even though convincing studies using dN/dS ratios are limited, studies using other genetic parameters, along with the frequent transitions toward selfing in plants and the comparatively low number of obligate selfers, show a clear picture (Wright et al. 2013; Cheptou 2019). In most lineages, selfers evolved due to short-term advantages such as reproductive assurance, but they face higher long-term extinction risks, consistent with the "dead-end" hypothesis of asexuals (Wright et al. 2013; Cheptou 2019). While this is in accordance with the observed elevated extinction and speciation rates in selfers, it is important to note that the ability to reproduce and colonize at low density may enhance their survival (Wright et al. 2013). Therefore, lineage-specific ecological and demographic factors may play an important role in the persistence and extinction of selfing lineages, even with clear signs of deleterious effects.

Hybridization and Polyploidy

The large majority of asexual species arise from past hybridization events and, in many cases, subsequent backcrossing (Simon et al. 2003; Hörandl 2024). These hybridization events frequently lead to polyploidization, resulting in a predominance of polyploid asexual taxa while their sexual relatives remain mostly diploid. Hybridization and polyploidization also generate elevated heterozygosity, retention of divergent alleles, structural genomic rearrangements and the presence of multiple homologous gene copies. Such features can enhance evolutionary potential by providing genetic redundancy and buffering deleterious mutations, but they also complicate genome evolution by creating complex patterns of allelic divergence and gene expression (Blanc-Mathieu et al. 2017; Janko et al. 2021).

High levels of heterozygosity in polyploids introduce additional methodological challenges. Mutations typically arise in the heterozygous state, making it difficult to accurately separate alleles and assess mutation rates or patterns. This complexity can obscure phylogenetic reconstruction, population genetic inference and analyses of adaptive evolution (Kajitani et al. 2014; Bourke et al. 2018). Consequently, the evolutionary trajectories of asexual polyploids cannot be fully understood without considering both the historical hybridization events that generated them and the genomic consequences that persist in these lineages.

Hybrids are often comparatively young and therefore, although they may experience relaxed selection, the relative short time span may not allow to detect changes in dN/dS ratios (Pellino et al. 2013). This, along with other life history traits, in particular facultative sexuality, may explain the varying effects of the different types of asexuality on dN/dS ratios (Hodac et al. 2019). In the majority of the taxa included in this review, asexuals are of hybrid origin (12 out of 20). When only investigating apo- and automicts, this number increases to 8 out of 12 (Table 1). Overall, again, no clear pattern of dN/dS ratios occurs in species/lineages of hybrid origin.

Models of dN/dS Ratios in Comparative Genomics

Models estimating the dN/dS ratio face several limitations that can bias selection inference (Del Amparo and Arenas, 2022). (i) In population-level data, unfixed mutations may be treated as fixed substitutions, leading to overestimation of dN/dS ratios. Using the ratio of nonsynonymous to synonymous polymorphisms (pN/pS) and/or the McDonald–Kreitman test provides a more appropriate assessment of ongoing selection (Nei and Li 1979; McDonald and Kreitman 1991). (ii) Recombination and gene conversion can produce genetic fragments with distinct evolutionary histories, inflating site-specific dN/dS. This bias can be alleviated by partitioning alignments into recombinant fragments (Anisimova et al. 2003; Del Amparo and Arenas, 2022). (iii) Heterogeneous codon frequencies among sites, caused by structural, functional or synonymous codon bias, can result in underestimation of dN/dS ratios. This can be mitigated by models that explicitly account for site-specific codon frequencies (e.g. CodABC; Arenas et al. 2015) or by partitioning sites with similar codon usage (Del Amparo and Arenas, 2022). (iv) Temporal variation in base composition (nonstationary GC content) can bias estimates, as low GC content inflates and high GC content deflates dN/dS ratios: Branch-specific dN/dS estimation can reduce this effect, although limited branch information may lower precision (Gueguen and Duret 2017; Del Amparo and Arenas, 2022). (v) Poor alignment quality, such as inclusion of nonhomologous positions, may artificially inflate dN/dS ratio; therefore, ensuring high-quality alignments is crucial to reliably estimate dN/dS ratios (Jordan and Goldman 2012). (vi) Assuming a constant synonymous substitution rate across sites may result in overestimating dN/dS ratios. Models such as the ones included in Hypotheses Testing Using Phylogenies (HyPhy) (Pond et al. 2004) or phylogenetic analyses using maximum likelihood (PAML) (Yang 2007), selecting the best-fitting substitution model using criteria such as AIC or BIC, can improve estimation accuracy (Del Amparo and Arenas, 2022). Together, these factors highlight the need for careful dataset curation, model selection and methodological adjustments when interpreting dN/dS ratios. Further **neutral selection** (dN/dS $\sim$ 1) cannot be distinguished from varying dN on a site or branch (Kryazhimskiy and Plotkin 2008). Similarly, most models cannot distinguish adaptive from relaxed selection when the dN/dS ratio is above one (Lahti et al. 2009; Wertheim et al. 2014).

Beyond these methodological concerns, models estimating dN/dS ratios also overlook key biological complexities, such as gene- and tissue-specific selection patterns, correlations between evolutionary rates and gene expression levels, and the effects of epistasis (Kimura 1983; Breen et al. 2012). Lastly, nonsynonymous substitutions are not always deleterious, as amino-acid changes are not equally detrimental as they may share physicochemical properties (James and Lascoux 2025), and this argues for further analyses (Weber and Whelan 2019). Hybridization poses another challenge. If asexual hybrids and only one of their sexual parent species are compared, species-specific single-nucleotide polymorphisms will appear as nonsynonymous substitutions in the hybrid (Paetzold et al. 2022).

Most studies reviewed here employed some variant of the PAML branch or branch-site models

(Zhang et al. 2005; Yang 2007). Branch models test for significant differences in dN/dS ratios between sexual and asexual taxa/lineages using likelihood ratio tests. Branch-site models operate similarly, but add an additional layer of resolution by classifying codons (sites) into categories based on their dN/dS ratios, reflecting the type of selection (i.e. positive, neutral or purifying) acting on them.

In general, branch models are relatively simplistic and detect shifts in selection only at the gene level, not at the codon level (Yang and Nielsen 2002), while branch-site models provide a more detailed view at the codon level. However, they are constrained by their reliance on prespecified site categories. These limitations can lead to false positives as well as failures to detect genuine positive selection. Moreover, these models frequently fail to distinguish between relaxed and positive selection, because they combine all codons from the selected branches, meaning that positive selection occurring in some branches may be masked by purifying selection in others (Pond et al. 2004).

The recently developed dN/dS-H framework addresses some of these limitations by incorporating site-specific heterogeneity into the estimation of selection pressures (Gu, 2022). By adjusting the neutral expectation for heterogeneity across codons, dN/dS-H can better distinguish between nearly neutral, purifying, and positive selection, reducing false positives and improving detection of adaptive evolution even when selection is uneven across sites.

The HyPhy suite (Pond et al. 2004) offers alternatives that can mitigate some of the limitations of PAML branch and branch-site models. This software suite implements several codon-level tests that make fewer assumptions about site categories or branch structure. For example, aBSREL (adaptive Branch-Site Random Effects Likelihood) is a flexible alternative to traditional PAML branch-site models (Smith et al. 2015). Rather than putting codons into strict categories, aBSREL allows dN/dS ratios to differ across branches and codons, leading to less bias and more nuanced results. Importantly, however, aBSREL cannot distinguish between purifying and relaxed selection. Therefore, a nonsignificant aBSREL result does not mean that there are no differences in dN/dS ratios among branches and sites. An instructive example for the usage of aBSREL as a supplementary tool is found in the study on whiptail lizards by Maldonado et al. (2022). They used aBSREL to identify possible adaptive selection in mitochondrial genes in order to distinguish it from relaxed selection.

Further, BUSTED (Branch-site Unrestricted Statistical Test for Episodic Diversification) is a method implemented in HyPhy for detecting gene-wide evidence of episodic positive selection at one or more sites along branches (Murrell et al. 2015). This approach summarizes selection at the gene level, helping to determine whether parts of a gene have experienced episodic diversifying selection in particular lineages. For example, BUSTED can be useful for testing whether genes in asexual lineages show episodic bursts of positive selection, possibly reflecting lineage-specific adaptation or compensatory evolution after the loss of recombination relative to their sexual counterparts. It is important to note that this approach is more suitable for recently transitioned asexuals, as ancient asexuals are expected to have already fixed important nondeleterious substitutions. In their original study, Murrell et al. (2015) identified episodes of positive selection in certain primate genes using this method.

Another model implemented in HyPhy is fixed effects likelihood (FEL), which estimates dN/dS at each codon site, thereby detecting site-specific selection without the need to preclassify codon types (Pond and Frost 2005). A modification of this framework, Contrast-FEL, is especially useful for investigating differences between sexual and asexual relatives as it includes the option to define branch sets (Pond et al. 2021). The model compares selective pressures at each codon site across these sets, identifying positions where the strength or mode of selection differs significantly. This approach avoids the averaging effects of traditional branch-site models and allows to directly test hypotheses such as whether asexuality is associated with relaxed purifying selection or shifts in adaptive pressures. While not peer reviewed, the study of Magpali et al. (2020) identified sites with diverging dN/dS ratios in marine and freshwater dolphins using Contrast-FEL.

Complementary to FEL, MEME (Mixed Effects Model of Evolution) is designed to detect episodic positive selection (Murrell et al. 2012). FEL assumes that the strength and type of selection at a given site are the same across all branches of a phylogenetic tree. Thereby, it treats each codon as if it experiences the same evolutionary pressures in all species. On the other hand, MEME allows selection to differ between branches, i.e. a codon can be positively selected in some lineages while being neutral or negatively selected in others. Because of this flexibility, MEME is especially powerful for detecting adaptive changes that are specific to certain lineages or temporary, which FEL might miss. Its extension, Contrast-MEME, allows to test whether such episodic selection events occur preferentially in one branch set relative to another, e.g. in sexual versus asexual lineages. By explicitly contrasting branch types, Contrast-MEME provides a nuanced view of whether adaptive events are unevenly distributed across, for example reproductive strategies, complementing the site-focused insights of Contrast-FEL.

Using MEME, Murrell et al. (2012) identified sites under positive selection in HIV-1 and influenza surface proteins, highlighting lineage-specific bursts of adaptation.

RELAX is another model implemented in HyPhy (Wertheim et al. 2014). It is particularly effective for distinguishing adaptive selection from relaxed purifying selection, a distinction that is often blurred in other dN/dS ratio-based tests. Rather than only testing if dN/dS ratios are elevated, RELAX estimates whether the intensity of selection has been strengthened or weakened along predefined sets of branches. For example, in the context of sexual versus asexual relatives, RELAX can test whether asexual lineages experience a general relaxation of purifying selection, consistent with reduced efficacy of selection in the absence of recombination or whether certain lineages show intensified selection. This makes RELAX a powerful complement to methods such as FEL, MEME, aBSREL and the traditional PAML-like models, as it directly addresses one of the key biological questions that arises when comparing the evolution of sexual and asexual species/lineages. Maldonado et al. (2022) used RELAX in combination with the PAML branch-site model and aBSREL to test whether increased dN/dS ratios in asexual species are due to adaptive or relaxed selection, identifying them to be caused by relaxed selection.

While no method is entirely free from assumptions, HyPhy tests are generally considered less prone to false positives than PAML models, particularly those arising from rigid site classifications or branch-based averaging (Pond and Frost, 2005). Together, these approaches offer complementary perspectives and, when used in combination, can provide a more nuanced view of selection, especially in complex evolutionary scenarios such as the divergence between sexual and asexual lineages.

Although PAML branch and branch-site models remain widely used and have proven highly valuable for many applications, incorporating more flexible frameworks, such as those implemented in HyPhy, can enhance interpretability and robustness. The comparatively little use of HyPhy methods may reflect citation bias and a perception of greater methodological complexity compared to PAML models rather than limitations in their utility. Ultimately, selecting the appropriate model depends on the biological question and data structure, with combining approaches yielding the most comprehensive insights into molecular evolution (Table 2).

Meta-Analysis of dN/dS Ratios Across Studies

To assess how strongly dN/dS ratios differ between sexual and asexual relatives across taxa, we compared published

Table 2 Overview of dN/dS ratio tests, their features, and the evolutionary question they may answer.

Method	Key features	Evolutionary question
PAML branch model	Compares dN/dS across predefined branches. The three-ratio model is a common approach. This model allows dN/dS ratios to differ between two or more terminal branches and internal branches.	Do lineage sets evolve under different selection across all sites?
PAML branch-site model	Detects codon-specific selection on specified **foreground branches** of interest versus **background branches**. Codons are classified into different categories based on selection and specified branch types	Are specific codons under positive selection in focal lineages?
aBSREL	Detects episodic positive selection on subsets of codons along individual branches. Can be either used as an exploratory tool or to investigate specific branches of interest	Do individual branches show episodic adaptive evolution on some codons?
BUSTED	Gene-level test for episodic selection. Identifies whether at least one codon experienced positive selection on at least one branch. Can be either used as an exploratory tool or to investigate specific branches of interest	Has a gene experienced bursts of adaptive evolution in any branch on any codon?
FEL/contrast-FEL	FEL estimates dN/dS at each codon across the phylogeny. Contrast-FEL compares codon-specific selection between branch sets and tests for significant differences	Do codons generally differ in their dN/dS ratios? Do codons experience different selection pressures in one lineage set versus another?
MEME/ contrast-MEME	MEME detects episodic positive selection at individual codons along subsets of branches. Contrast-MEME compares selection between predefined branch sets. And tests for significant differences	Do some codons experience episodic selection, while others do not? Do codons experience episodic selection in one lineage set versus another?
RELAX	Tests for relaxation or intensification of selection on predefined branch sets (test vs. reference) using the selection intensity parameter k. $k = 1$: no difference, $k > 1$: intensification of selection in test branches compared to reference branches, $k < 1$: relaxation of selection in test branches compared to reference branches	Is selection stronger or weaker in one set of branches compared to another?

All models which are not specified to be included in PAML are part of the HyPhy suite.

mean dN/dS estimates by calculating fold changes (mean dN/dS asexual/mean dN/dS sexual; A/S). We had to exclude many of the reviewed studies here due to insufficient data availability, potential biases arising from gene choice (e.g. RNA helicases in Kiefer et al. 2020), or biological confounding factors (e.g. Wang et al. 2025, which compared the distantly related Bambooideae and Pooideae, each encompassing multiple and overlapping reproductive modes). For studies reporting species-specific estimates (e.g. Ament-Velásquez et al. 2016), fold-change ranges were calculated rather than single values. Data for organelles were only available for animal mitochondria.

The remaining studies were grouped by reproductive mode (automixis, apomixis, selfing, and fissiparity/clonal growth) and by outcome (significantly higher dN/dS in asexuals, significantly higher in sexuals, or no significant difference; Fig. 2). Across nuclear genes, fold changes between asexual and sexual lineages were generally small (A/S: 0.89 to 1.18), regardless of reproductive mode or statistical significance. Even when nuclear dN/dS ratios differed significantly, effect sizes were modest, suggesting that these differences are unlikely to reflect genome-wide mutational decay associated with substantial fitness loss. Instead, they are more consistent with gene-specific selective pressures, as previously proposed (Brandt et al. 2017; Kiefer et al. 2020), which may also explain the heterogeneous and often ambiguous patterns reported across studies.

By contrast, mitochondrial genes exhibited substantially larger differences between reproductive modes. The greatest fold changes were observed in *Aspidoscelis* (A/S = 1.92), *P. antipodarum* (A/S = 1.85) and the mitochondrial genes of Oribatida (A/S = 1.48), showing significantly elevated mitochondrial dN/dS ratios in asexual lineages. Oribatid mites are of special significance, as the study by Brandt et al. (2017) also had a nuclear dN/dS ratio available, which alongside with all other nuclear genes shows little actual differences between sexuals and asexuals, despite these differences being significant. An apparent exception is *Trichogramma*, where differences were not statistically significant; however, this study reported species-specific estimates, yielding a fold-change range of 0.90 to 1.25, with the upper bound representing the third-highest mitochondrial difference across all taxa included. Thus, even in cases lacking statistical significance, mitochondrial effect sizes remain comparatively large.

Although this meta-analysis is limited in scope, the consistent contrast between nuclear and mitochondrial patterns is striking. While nuclear dN/dS ratios show limited and inconsistent divergence between sexual and asexual lineages, mitochondrial genes display a recurrent trend toward elevated dN/dS in asexuals. This pattern aligns well with the hypothesis proposed by Havird et al. (2015) that sex is maintained in part to

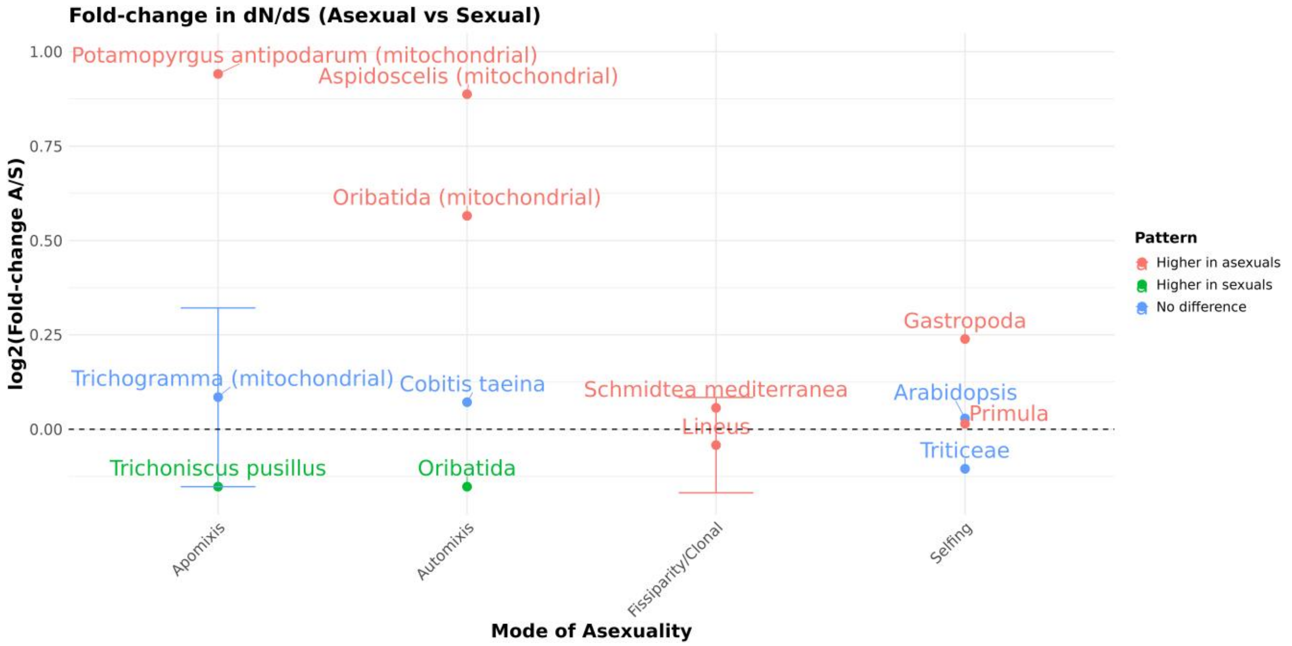

Fig. 2. Log2 fold-change in dN/dS between asexual and sexual lineages across multiple studies. Error bars show the range from minimum to maximum fold-change values when variability exists. Studies which observed significantly higher dN/dS ratios in asexuals are marked red, studies which observed significantly higher dN/dS ratios in sexuals are marked green, and studies observing no significant differences are marked blue. The dashed horizontal line at 0 represents equal dN/dS ratios in asexual and sexual lineages.

mitigate the accumulation of deleterious mutations in mitochondrial genomes through enhanced mito-nuclear coadaptation. Empirical studies across eukaryotes, including detailed analyses of nuclear-encoded organellar genes and organelle–nuclear interactions, are still needed to test this hypothesis. It is important to note that long-term asexuals must effectively circumvent this process to maintain fitness. Under this framework, the relatively strong and consistent mitochondrial signal observed across asexual taxa may represent a key component of the evolutionary costs of asexuality.

Conclusion

Overall, the results of our review suggest that studies using dN/dS ratios to compare asexual lineages with their sexual relatives do not reveal a consistent pattern. Among the 20 taxa reviewed, 12 showed at least partially increased dN/dS ratios in nonsexuals, six showed no significant differences, and two had slightly higher dN/dS ratios in sexuals. While this might suggest a general trend of higher dN/dS ratios in asexuals, seven of the 12 taxa with elevated ratios reproduce via selfing or fissiparity. All three fissiparous taxa and four of the five selfing taxa had higher dN/dS ratios. Relaxed purifying selection, reflected in elevated dN/dS ratios, therefore appears to be most pronounced in these reproductive modes, which resemble clonal growth. Moreover, heterozygosity does not seem to influence these ratios: selfers typically have low heterozygosity, whereas fissiparous lineages often maintain high heterozygosity, yet both consistently show elevated dN/dS ratios. This suggests that life-history traits and reproductive mode, rather than heterozygosity, drive the accumulation of nonsynonymous mutations.

Studies of apomictic and automictic asexuals showed even less consistent patterns. Among the eight apomictic taxa, three had increased dN/dS ratios in asexuals, four showed no significant differences, and one (Isopoda) had higher dN/dS ratios in sexuals. Similarly, of the four automictic taxa, two reported elevated dN/dS ratios in asexuals (though the results for *Daphnia pulex* should be interpreted with caution), one showed no significant differences, and one (Oribatida) exhibited slightly higher dN/dS ratios in sexuals.

Ecology may play an important role in impacting selection as it largely dictates the effective population size. This may explain why the only two taxa with elevated dN/dS ratios in sexual species, i.e. Oribatida and Isopoda, are both soil-dwelling detritivores. Studies using oribatid mites have shown that asexuals dominate in resource-rich habitats where they reach high densities, which may positively influence effective population size, enabling more efficient purifying selection (Maraun et al. 2012). While this is an interesting idea, more research is needed. Even though parthenogenesis is widespread in many soil organisms (Scheu and Drossel 2007), only a few have been thoroughly investigated using molecular methods.

Further, the inconclusive results are at least in part likely due to different genes examined. For example, essential functional genes directly involved in processes such as meiosis and gametogenesis are likely to be affected differently than other genes. Such highly conserved genes certainly behave differently from those under low selective pressure. Moreover, genes that are not influenced by mixis (such as those in the cytogenome) may show different patterns compared to genes that are affected by it. This is well represented in the mitochondrial genes included here, as they generally exhibit much higher dN/dS ratios in asexuals than in sexuals. These differences provide opportunities to investigate how, and to what extent, asexuality shapes the genome.

Studies reviewed here rarely address the beforementioned methodological limitations of dN/dS ratio models, such as possible recombinational fragments or heterogeneous codon frequencies. Further, simpler branch and branch-site models may fail to detect subtle selective differences that occur only at a few codons or along specific branches, potentially underestimating the complexity of evolutionary dynamics. Consequently, it is essential to choose models that align with the biological question and the structure of the data, and to complement traditional approaches with more sensitive or flexible frameworks. For example, site-level models such as FEL and MEME, or their contrast variants, can detect branch- or site-specific selection that simpler models might miss. Awareness of these methodological nuances allows researchers to interpret results more accurately, avoid overgeneralizations and design studies that fully capture the genomic consequences of sexual versus asexual reproduction. Ultimately, careful model selection, combined with rigorous data preparation and appropriate comparative controls, strengthens the reliability and interpretability of dN/dS ratio analyses.

It is important to consider that all the asexual lineages persist at least for some period of time and therefore increased dN/dS ratios may not be associated with an immediate loss of fitness. To enable more robust analyses of the genomic consequences of asexuality, future studies should prioritize data comparability. Whole-genome sequences and carefully selected orthologs (e.g. large numbers of one-to-one orthologs) are essential to reduce gene-specific biases. The choice of dN/dS ratio models is also critical; PAML branch-site models may miss codon-specific differences that more sensitive approaches, such as contrast-FEL, can detect. While existing studies often include evolutionary replicates, future work must avoid comparing distantly related sexual and asexual lineages and should not infer the effects of asexuality without appropriate sexual controls.

This review highlights the importance of considering the diversity of reproductive modes, methodology used and lineage-specific traits when assessing general consequences of asexuality. Studies often cite results from unrelated taxa with very different modes of asexual reproduction, using different dN/dS ratio models without accounting for these critical differences. It further shows the urgent need for the investigation of different taxa with asexual lineages across the tree of life using molecular and cytological methods to assess and analyze the consequences of their reproductive mode.

Glossary

Adaptive selection
A form of positive selection in which genetic changes give organisms an advantage in adapting to their environment.

Adventitious embryony/sporophytic apomixis
A type of plant apomixis where embryos form from somatic (nonreproductive) cells of the ovule, such as nucellar or integument cells, instead of from the egg cell, producing clonal offspring.

Apomixis (ameiotic/mitotic)
Asexual reproduction where an unreduced egg cell develops without meiosis or fertilization, generating offspring genetically identical to the parent.

Apospory
A form of gametophytic apomixis in plants in which a gametophyte develops directly from somatic cells of the sporophyte, bypassing sexual spore formation. The egg cell develops parthenogenetically.

Automixis
Asexual reproduction in which meiotic changes restore the egg cell's ploidy without sperm contribution, producing nonclonal but uniparental offspring.

Background branches
Branches in evolutionary analyses that serve as a reference group, against which selection on foreground branches is compared.

Clonal growth
Nonsexual reproduction through budding or fragmentation of somatic tissue, producing genetically identical offspring while the parent remains intact.

Cyclical parthenogenesis
A reproductive strategy alternating between asexual reproduction (parthenogenesis) and sexual reproduction, usually triggered by environmental conditions.

Diplospory
A type of gametophytic apomixis where an unreduced gametophyte develops directly from a megaspore mother cell, bypassing or modifying meiosis. The egg cell develops parthenogenetically.

dN/dS ratio
The ratio of nonsynonymous (amino acid-changing) to synonymous (silent) nucleotide substitutions is used to infer the type and strength of natural selection on protein-coding genes.

Fissiparity
A reproductive mode in which an organism splits into two or more parts, each regenerating into a complete individual, producing somatic clones without meiosis or fertilization.

Foreground branches
Branches in evolutionary analyses specifically tested for adaptive or relaxed selection.

Gametophytic apomixis
Asexual reproduction in plants where an embryo develops from an unreduced cell within an embryo sac, bypassing fertilization.

Gynogenesis
A form of asexual reproduction in which an egg develops after being stimulated by a sperm, but the sperm contributes no genetic material.

Neutral selection
Occurs when $dN/dS \approx 1$, suggesting amino acid–changing mutations are neither favored nor disfavored. True neutrality is rare and can be hard to demonstrate.

Outcrossing
Reproduction involving fusion of gametes from two different individuals, following meiosis and crossing-over to produce genetically diverse offspring.

Parthenogenesis
Asexual reproduction where offspring develop from egg cells without paternal genetic contribution, either because fertilization does not occur or the paternal genome is excluded.

Positive selection
Occurs when dN/dS > 1, indicating an excess of amino acid–changing mutations, often reflecting adaptive or relaxed purifying selection.

Purifying selection
Occurs when dN/dS < 1, indicating removal of deleterious mutations to maintain gene function.

Relaxed selection
A reduction in selective pressure, often involving weakened purifying selection compared to another lineage or ancestral state.

Selfing (self-fertilization)
Reproduction via fusion of gametes from the same individual, including normal meiosis and crossing-over.

Sex
The alternation of meiosis, producing reduced gametes either directly or via gametophytes and their fusion. It involves two types of recombination: intrachromosomal (crossing-over during meiosis) and interchromosomal (gametic recombination).

Sites
Individual codons in a gene are used in molecular evolution analyses (e.g. dN/dS models) to assess selective pressure.

Supplementary Material

Supplementary material is available at *Genome Biology and Evolution* online.

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Author Contributions

Finn L. Klemp (Conceptualization, Literature search, Writing—original draft, Figures), Elvira Hörandl (Literature search, Writing—original draft, Writing—review & editing), Mark Maraun (Conceptualization, Literature search, Writing—review & editing), Stefan Scheu (Conceptualization, Literature search, Writing—review & editing), Bastian Heimburger (Conceptualization, Writing—review & editing).

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

No data were generated for this review. All results presented here are published and cited appropriately.

Literature Cited

Ament-Velásquez SL, et al. Population genomics of sexual and asexual lineages in fissiparous ribbon worms (Lineus, Nemertea): hybridization, polyploidy and the Meselson effect. Mol Ecol. 2016:25:3356–3369. https://doi.org/10.1111/mec.13717.

Anisimova , M, Nielsen R, Yang Z, Effect of recombination on the accuracy of the likelihood method for detecting positive selection at amino acid sites. Genetics. 2003:164:1229–1236. https://doi.org/10.1093/genetics/164.3.1229.

Archetti M. Evidence from automixis with inverted meiosis for the maintenance of sex by loss of complementation. J Evol Biol. 2021:35:40–50. https://doi.org/10.1111/jeb.13975.

Arenas M, Lopes JS, Beaumont MA, Posada D. CODABC: a computational framework to coestimate recombination, substitution, and molecular adaptation rates by approximate Bayesian computation. Mol Biol Evol. 2015:32:1109–1112. https://doi.org/10.1093/molbev/msu411.

Asker SE, Jerling L. Mechanisms of Apomixis. In: Apomixis in plants. CRC Press; 1992. p. 49–75. https://doi.org/10.1201/9781315137537.

Baker HG. Self-compatibility and establishment after "long-distance" dispersal. Evolution. 1955:9:347–349. https://doi.org/10.1111/j.1558-5646.1955.tb01544.x.

Barrett SCH. The evolution of plant sexual diversity. Nat Rev Genet. 2002:3:274–284. https://doi.org/10.1038/nrg776.

Bast J, et al. Consequences of asexuality in natural populations: insights from stick insects. Mol Biol Evol. 2018:35:1668–1677. https://doi.org/10.1093/molbev/msy058.

Bell G. The masterpiece of nature: the evolution and genetics of sexuality. University of California Press; 1982.

Bierne J. 1970. Recherches sur la différenciation sexuelle au cours de l'ontogenèse et de la régénération chez le némertien Lineus ruber (Müller) [Doktoral Dissertation].

Bierne J, Tarpin M, Vernet G, A reassessment of the systematics and a proposal for the phylogeny of some cosmopolitan *Lineus* species (Nemertea). Hydrobiologia. 1993:266:159–168.

Blanc CS, Delattre M, Van Doninck K. Cracking the meiotic secrets behind animals asexuality when cytology seduces genomics to decode asexual meiosis. J Evol Biol. 2025:38:832–850. https://doi.org/10.1093/jeb/voaf045.

Blanc-Mathieu R, et al. Hybridization and polyploidy enable genomic plasticity without sex in the most devastating plant-parasitic nematodes. PLoS Genet. 2017:13:e1006777. https://doi.org/10.1371/journal.pgen.1006777.

Bourke PM, Voorrips RE, Visser RGF, Maliepaard C. Tools for genetic studies in experimental populations of polyploids. Front Plant Sci. 2018:9:513. https://doi.org/10.3389/fpls.2018.00513.

Brandeis M. New-age ideas about age-old sex: separating meiosis from mating could solve a century-old conundrum. Biol Rev Camb Philos Soc. 2017:93:801–810. https://doi.org/10.1111/brv.12367.

Brandt A, et al. Effective purifying selection in ancient asexual oribatid mites. Nat Commun. 2017:8:873. https://doi.org/10.1038/s41467-017-01002-8.

Brandt A, et al. No signal of deleterious mutation accumulation in conserved gene sequences of extant asexual hexapods. Sci Rep. 2019:9:5338. https://doi.org/10.1038/s41598-019-41821-x.

Brandt A, et al. Haplotype divergence supports long-term asexuality in the oribatid mite *Oppiella nova*. Proc Natl Acad Sci U S A. 2021: 118:e2101485118. https://doi.org/10.1073/pnas.2101485118.

Breen MS, Kemena C, Vlasov PK, Notredame C, Kondrashov FA. Epistasis as the primary factor in molecular evolution. Nature. 2012:490:535–538. https://doi.org/10.1038/nature11510.

Burgarella C, et al. Molecular evolution of freshwater snails with contrasting mating systems. Mol Biol Evol. 2015:32: 2403–2416. https://doi.org/10.1093/molbev/msv121.

Burgarella C, Glémin S. Population genetics and genome evolution of selfing species. Encyclopedia of Life Sciences. John Wiley & Sons, Ltd; 2017. p. 1–8. https://doi.org/10.1002/9780470015902.a0026804.

Burke NW, Bonduriansky R. Sexual conflict, facultative asexuality, and the true paradox of sex. Trends Ecol Evol. 2017:32: 646–652. https://doi.org/10.1016/j.tree.2017.06.002.

Byers DL, Waller DM. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. Annu Rev Ecol Syst. 1999:30:479–513. https://doi.org/10.1146/annurev.ecolsys.30.1.479.

Charlesworth B. Effective population size and patterns of molecular evolution and variation. Nat Rev Genet. 2009:10:195–205. https://doi.org/10.1038/nrg2526.

Charlesworth B, Charlesworth D. The genetic basis of inbreeding depression. Genet Res (Camb). 1999:74:329–340. https://doi.org/10.1017/s0016672399004152.

Charlesworth B, Morgan MT, Charlesworth D. The effect of deleterious mutations on neutral molecular variation. Genetics. 1993:134:1289–1303. https://doi.org/10.1093/genetics/134.4.1289.

Cheptou P. Does the evolution of self-fertilization rescue populations or increase the risk of extinction? Ann Bot. 2019:123: 337–345. https://doi.org/10.1093/aob/mcy144.

Cleland RE. Oenothera, cytogenetics and evolution. Kew Bull. 1974:29:237. https://doi.org/10.2307/4108389.

Coe WR. Regeneration in nemerteans. J Exp Zool. 1929:54: 411–459. https://doi.org/10.1002/jez.1400540304.

Crick FHC, Barnett L, Brenner S, Watts-Tobin RJ. General nature of the genetic code for proteins. Nature. 1961:192:1227–1232. https://doi.org/10.1038/1921227a0.

Cutter AD, Wasmuth JD, Washington NL. Patterns of molecular evolution in caenorhabditis preclude ancient origins of selfing. Genetics. 2008:178:2093–2104. https://doi.org/10.1534/genetics.107.085787.

Del Amparo R, Arenas M. Consequences of substitution model selection on protein ancestral sequence reconstruction. Mol Biol Evol. 2022:39:msac144. https://doi.org/10.1093/molbev/msac144.

Edwards DM, et al. Avoiding organelle mutational meltdown across eukaryotes with or without a germline bottleneck. PLoS Biol. 2021:19:e3001153. https://doi.org/10.1371/journal.pbio.3001153.

Engelstädter J. Asexual but not clonal: evolutionary processes in automictic populations. Genetics. 2017:206:993–1009. https://doi.org/10.1534/genetics.116.196873.

Escobar JS, et al. An integrative test of the dead-end hypothesis of selfing evolution in triticeae (Poaceae). Evolution. 2010:64: 2855–2872. https://doi.org/10.1111/j.1558-5646.2010.01045.x.

Eyre-Walker A, Keightley PD. The distribution of fitness effects of new mutations. Nat Rev Genet. 2007:8:610–618. https://doi.org/10.1038/nrg2146.

Felsenstein J. The evolutionary advantage of recombination. Genetics. 1974:78:737–756. https://doi.org/10.1093/genetics/78.2.737.

Glémin S. How are deleterious mutations purged? Drift versus nonrandom mating. Evolution. 2003:57:2678–2687. https://doi.org/10.1111/j.0014-3820.2003.tb01512.x.

Glémin S. Balancing selection in self-fertilizing populations. Evolution. 2021:75:1011–1029. https://doi.org/10.1111/evo.14194.

Glémin S, François CM, Galtier N. Genome evolution in outcrossing vs. selfing vs. asexual species. In: Anisimova M, editors. Evolutionary genomics. Methods in Molecular Biology; 2019. p. 331–369. https://doi.org/10.1007/978-1-4939-9074-0_11.

Gravel S. When is selection effective? Genetics. 2016:203:451–462. https://doi.org/10.1534/genetics.115.184630.

Green RF, Noakes DL. Is a little bit of sex as good as a lot? J Theor Biol. 1995:174:87–96. https://doi.org/10.1006/jtbi.1995.0081.

Grosberg RK, Strathmann RR. The evolution of multicellularity: a minor major transition? Annu Rev Ecol Evol Syst. 2007:38:621–654. https://doi.org/10.1146/annurev.ecolsys.36.102403.114735.

Gu X. DN/DS-H, a new test to distinguish different selection modes in protein evolution and cancer evolution. J Mol Evol. 2022:90: 342–351. https://doi.org/10.1007/s00239-022-10064-2.

Guéguen L, Duret L. Unbiased estimate of synonymous and nonsynonymous substitution rates with nonstationary base composition. Mol Biol Evol. 2017:35:734–742. https://doi.org/10.1093/molbev/msx308.

Haudry A, et al. Mating system and recombination affect molecular evolution in four Triticeae species. Genet Res (Camb). 2008:90:97–109. https://doi.org/10.1017/s0016672307009032.

Havird JC, Hall MD, Dowling DK. The evolution of sex: a new hypothesis based on mitochondrial mutational erosion. BioEssays. 2015:37:951–958. https://doi.org/10.1002/bies.201500057.

Hebert PDN, Crease TJ. Clonal coexistence in Daphnia pulex (Leydig): another planktonic paradox. Science. 1980:207: 1363–1365. https://doi.org/10.1126/science.207.4437.1363.

Heethoff M, Norton RA, Scheu S, Maraun M. Parthenogenesis in oribatid mites (Acari, Oribatida): evolution without sex. Lost Sex. Springer International Publishing; 2009. p. 241–257. https://doi.org/10.1007/978-90-481-2770-2_12.

Henry L, Schwander T, Crespi BJ. Deleterious mutation accumulation in asexual *Timema* stick insects. Mol Biol Evol. 2011:29: 401–408. https://doi.org/10.1093/molbev/msr237.

Hershberg R, Petrov DA. Selection on codon bias. Annu Rev Genet. 2008:42:287–299. https://doi.org/10.1146/annurev.genet.42.110807.091442.

Hill WG, Robertson A. The effect of linkage on limits to artificial selection. Genet Res (Camb). 1966:8:269–294. https://doi.org/10.1017/s0016672300010156.

Hiruta C, Nishida C, Tochinai S. Abortive meiosis in the oogenesis of parthenogenetic Daphnia pulex. Chromosome Res. 2010:18: 833–840. https://doi.org/10.1007/s10577-010-9159-2.

Hodač L, Klatt S, Hojsgaard D, Sharbel TF, Hörandl E. A little bit of sex prevents mutation accumulation even in apomictic polyploid plants. BMC Evol Biol. 2019:19:170. https://doi.org/10.1186/s12862-019-1495-z.

Hojsgaard D, Hörandl E. A little bit of sex matters for genome evolution in asexual plants. Front Plant Sci. 2015:6:82. https://doi.org/10.3389/fpls.2015.00082.

Hollister JD, et al. Recurrent loss of sex is associated with accumulation of deleterious mutations in Oenothera. Mol Biol Evol. 2015:32:896–905. https://doi.org/10.1093/molbev/msu345.

Hörandl E. A combinational theory for maintenance of sex. Heredity (Edinb). 2009:103:445–457. https://doi.org/10.1038/hdy.2009.85.

Hörandl E, et al. Genome evolution of asexual organisms and the paradox of sex in eukaryotes. In: Evolutionary biology—a transdisciplinary approach. Springer International Publishing; 2020. p. 133–167. https://doi.org/10.1007/978-3-030-57246-4_7.

Hörandl E. Apomixis and the paradox of sex in plants. Ann Bot. 2024:134:1–18. https://doi.org/10.1093/aob/mcae044.

Hughes AL. Looking for Darwin in all the wrong places: the misguided quest for positive selection at the nucleotide sequence level. Heredity (Edinb). 2007:99:364–373. https://doi.org/10.1038/sj.hdy.6801031.

Hughes RN. A functional biology of clonal animals. J Anim Ecol. 1989:59:797. https://doi.org/10.2307/4898.

Huxley TH. Upon animal individuality. Royal Institution of Great Britain; 1852.

Igic B, Kohn JR. The distribution of plant mating systems: study bias against obligately outcrossing species. Evolution. 2006:60:1098–1103. https://doi.org/10.1111/j.0014-3820.2006.tb01186.x.

James JE, Lascoux M. Amino acid properties, substitution rates, and the nearly neutral theory. Genome Biol Evol. 2025:17:evaf025. https://doi.org/10.1093/gbe/evaf025.

Janko K, et al. Genome fractionation and loss of heterozygosity in hybrids and polyploids: mechanisms, consequences for selection, and link to gene function. Mol Biol Evol. 2021:38:5255–5274. https://doi.org/10.1093/molbev/msab249.

Jarne P, Charlesworth D. The evolution of the selfing rate in functionally hermaphrodite plants and animals. Annu Rev Ecol Syst. 1993:24:441–466. https://doi.org/10.1146/annurev.es.24.110193.002301.

Jaron KS, et al. Genomic features of parthenogenetic animals. J Hered. 2021:112:19–33. https://doi.org/10.1093/jhered/esaa031

Jaron KS, et al. Convergent consequences of parthenogenesis on stick insect genomes. Sci Adv. 2022:8:eabg3842. https://doi.org/10.1126/sciadv.abg3842.

Jordan G, Goldman N. The effects of alignment error and alignment filtering on the sitewise detection of positive selection. Mol Biol Evol. 2012:29:1125–1139. https://doi.org/10.1093/molbev/msr272.

Judson OP, Normark BB. Ancient asexual scandals. Trends Ecol Evol. 1996:11:41–46. https://doi.org/10.1016/0169-5347(96)81040-8.

Kajitani R, et al. Efficient de novo assembly of highly heterozygous genomes from whole-genome shotgun short reads. Genome Res. 2014:24:1384–1395. https://doi.org/10.1101/gr.170720.113.

Kershenbaum S, Espejo MJ, Griffin A, Aboobaker A. 2024. Gene conversion limits the cost of asexuality in somatically immortal worms [preprint]. bioRxiv (Cold Spring Harbor Laboratory). https://doi.org/10.1101/2023.03.20.533553.

Khakhlova O, Bock R. Elimination of deleterious mutations in plastid genomes by gene conversion. Plant J. 2006:46:85–94. https://doi.org/10.1111/j.1365-313x.2006.02673.x.

Kiefer M, et al. Gene function rather than reproductive mode drives the evolution of RNA helicases in sexual and apomictic Boechera. Genome Biol Evol. 2020:12:656–673. https://doi.org/10.1093/gbe/evaa078.

Kimura M. The neutral theory of molecular evolution. Cambridge University Press; 1983. https://doi.org/10.1017/cbo9780511623486.

Kočí J, et al. No evidence for accumulation of deleterious mutations and fitness degradation in clonal fish hybrids: abandoning sex without regrets. Mol Ecol. 2020:29:3038–3055. https://doi.org/10.1111/mec.15539.

Kondrashov AS. Deleterious mutations and the evolution of sexual reproduction. Nature. 1988:336:435–440. https://doi.org/10.1038/336435a0.

Kryazhimskiy S, Plotkin JB. The population genetics of DN/DS. PLoS Genet. 2008:4:e1000304. https://doi.org/10.1371/journal.pgen.1000304.

Lahti DC, et al. Relaxed selection in the wild. Trends Ecol Evol. 2009:24:487–496. https://doi.org/10.1016/j.tree.2009.03.010.

Lande R, Schemske DW. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evolution. 1985:39:24–40. https://doi.org/10.1111/j.1558-5646.1985.tb04077.x.

Landis JB, et al. Impact of whole-genome duplication events on diversification rates in angiosperms. Am J Bot. 2018:105:348–363. https://doi.org/10.1002/ajb2.1060.

Lehtonen J, Jennions MD, Kokko H. The many costs of sex. Trends Ecol Evol. 2012:27:172–178. https://doi.org/10.1016/j.tree.2011.09.016.

Lively CM. Evidence from a New Zealand snail for the maintenance of sex by parasitism. Nature. 1987:328:519–521. https://doi.org/10.1038/328519a0.

Lovell JT, Williamson RJ, Wright SI, McKay JK, Sharbel TF. Mutation accumulation in an asexual relative of Arabidopsis. PLoS Genet. 2017:13:e1006550. https://doi.org/10.1371/journal.pgen.1006550.

Lunt DH. Genetic tests of ancient asexuality in Root Knot Nematodes reveal recent hybrid origins. BMC Evol Biol. 2008:8:194. https://doi.org/10.1186/1471-2148-8-194.

Lynch M, Bürger R, Butcher D, Gabriel W. The mutational meltdown in asexual populations. J Hered. 1993:84:339–344. https://doi.org/10.1093/oxfordjournals.jhered.a111354.

Magpali L, Freitas L, Ramos EKS, Nery MF. 2020. Molecular evolution in dolphin hearing genes: different evolutionary pathways for marine and freshwater echolocation? In https://prp.unicamp.br/inscricao-congresso/resumos/2020P17354A29318O4821.pdf. Presented at the XXVIII Congresso de Iniciação Científica da UNICAMP, Campinas, SP, Brazil.

Maldonado JA, Firneno TJ, Hall AS, Fujita MK. Parthenogenesis doubles the rate of amino acid substitution in whiptail mitochondria. Evolution. 2022:76:1434–1442. https://doi.org/10.1111/evo.14509.

Maraun M, Norton RA, Ehnes RB, Scheu S, Erdmann G. Positive correlation between density and parthenogenetic reproduction in oribatid mites (Acari) supports the structured resource theory of sexual reproduction. Evol Ecol Res. 2012:14:311–323. http://www.Evolutionary-Ecology.com/issues/v14/n03/ggar2744.pdf

Maynard Smith J. What use is sex? J Theor Biol. 1971:30:319–335. https://doi.org/10.1016/0022-5193(71)90058-0.

McDonald JH, Kreitman M. Adaptive protein evolution at the Adh locus in Drosophila. Nature. 1991:351:652–654. https://doi.org/10.1038/351652a0.

Mogie M. The evolution of asexual reproduction in plants. Chapman and Hall; 1992.

Mower JP, et al. Horizontal acquisition of multiple mitochondrial genes from a parasitic plant followed by gene conversion with host mitochondrial genes. BMC Biol. 2010:8:150. https://doi.org/10.1186/1741-7007-8-150.

Muller H. The relation of recombination to mutational advance. Mutat Res. 1964:1:2–9. https://doi.org/10.1016/0027-5107(64)90047-8.

Murrell B, et al. Detecting individual sites subject to episodic diversifying selection. PLoS Genet. 2012:8:e1002764. https://doi.org/10.1371/journal.pgen.1002764.

Murrell B, et al. Gene-wide identification of episodic selection. Mol Biol Evol. 2015:32:1365–1371. https://doi.org/10.1093/molbev/msv035.

Nei M, Gojobori T. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Mol Biol Evol. 1986:3:418–426. https://doi.org/10.1093/oxfordjournals.molbev.a040410.

Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA. 1979:76:5269–5273. https://doi.org/10.1073/pnas.76.10.5269.

Nei M, Maruyama T, Chakraborty R. The bottleneck effect and genetic variability in populations. Evolution. 1975:29:1. https://doi.org/10.2307/2407137.

Neiman M, Hehman G, Miller JT, Logsdon JM, Taylor DR. Accelerated mutation accumulation in asexual lineages of a freshwater snail. Mol Biol Evol. 2009:27:954–963. https://doi.org/10.1093/molbev/msp300.

Neiman M, Lively CM, Meirmans S. Why sex? A pluralist approach revisited. Trends Ecol Evol. 2017:32:589–600. https://doi.org/10.1016/j.tree.2017.05.004.

Neiman M, Sharbel TF, Schwander T. Genetic causes of transitions from sexual reproduction to asexuality in plants and animals. J Evol Biol. 2014:27:1346–1359. https://doi.org/10.1111/jeb.12357.

Nirenberg M, Leder P. RNA codewords and protein synthesis. Science. 1964:145:1399–1407. https://doi.org/10.1126/science.145.3639.1399.

Ohta T. Slightly deleterious mutant substitutions in evolution. Nature. 1973:246:96–98. https://doi.org/10.1038/246096a0.

Otto SP, Gerstein A. Why have sex? The population genetics of sex and recombination. Biochem Soc Trans. 2006:34:519–522. https://doi.org/10.1042/bst0340519.

Otto SP, Lenormand T. Resolving the paradox of sex and recombination. Nat Rev Genet. 2002:3:252–261. https://doi.org/10.1038/nrg761.

Öztoprak H, et al. Chromosome-scale genome dynamics reveal signatures of independent haplotype evolution in the ancient asexual mite Platynothrus peltifer. Sci Adv. 2025:11: eadn0817. https://doi.org/10.1126/sciadv.adn0817.

Paland S, Lynch M. Transitions to asexuality result in excess amino acid substitutions. Science. 2006:311:990992. https://doi.org/10.1126/science.1118152.

Pätzold C, Barke BH, Hörandl E. Evolution of transcriptomes in early-generation hybrids of the apomictic ranunculus auricomus complex (Ranunculaceae). Int J Mol Sci. 2022:23. https://doi.org/10.3390/ijms232213881.

Payne BL, Alvarez-Ponce D. Higher rates of protein evolution in the self-fertilizing plant Arabidopsis thaliana than in the out-crossers Arabidopsis lyrata and Arabidopsis halleri. Genome Biol Evol. 2018:10:895–900. https://doi.org/10.1093/gbe/evy053.

Pellino M, et al. Asexual genome evolution in the apomictic *Ranunculus auricomus* complex: examining the effects of hybridization and mutation accumulation. Mol Ecol. 2013:22: 5908–5921. https://doi.org/10.1111/mec.12533.

Pond SLK, Frost SDW. Not so different after all: a comparison of methods for detecting amino acid sites under selection. Mol Biol Evol. 2005:22:1208–1222. https://doi.org/10.1093/molbev/msi105.

Pond SLK, Frost SDW, Muse SV. Hyphy: hypothesis testing using phylogenies. Bioinformatics. 2004:21:676–679. https://doi.org/10.1093/bioinformatics/bti079.

Pond SLK, Wisotsky SR, Escalante A, Magalis BR, Weaver S. Contrast-FEL—a test for differences in selective pressures at individual sites among clades and sets of branches. Mol Biol Evol. 2021:38:1184–1198. https://doi.org/10.1093/molbev/msaa263.

Richards AJ. Plant breeding systems. 2nd ed. Chapmann and Hall; 1997.

Scheu S, Drossel B. Sexual reproduction prevails in a world of structured resources in short supply. Proc R Soc Lond B Biol Sci. 2007:274:1225–1231. https://doi.org/10.1098/rspb.2007.0040.

Schwander T, Crespi BJ. Multiple direct transitions from sexual reproduction to apomictic parthenogenesis intimemastick insects. Evolution. 2009:63:84–103. https://doi.org/10.1111/j.1558-5646.2008.00524.x.

Schwander T, Henry L, Crespi BJ. Molecular evidence for ancient asexuality in Timema stick insects. Curr Biol. 2011:21: 1129–1134. https://doi.org/10.1016/j.cub.2011.05.026.

Simon J, Delmotte F, Rispe C, Crease T. Phylogenetic relationships between parthenogens and their sexual relatives: the possible routes to parthenogenesis in animals. Biol J Linn Soc Lond. 2003:79:151–163. https://doi.org/10.1046/j.1095-8312.2003.00175.x.

Smith MD, et al. Less is more: an adaptive branch-site random effects model for efficient detection of episodic diversifying selection. Mol Biol Evol. 2015:32:1342–1353. https://doi.org/10.1093/molbev/msv022.

Suomalainen E, Saura A, Lokki J. Cytology and evolution in parthenogenesis. 44. CRC Press; 1987.

Taberly G. La cytologie de la parthenogenese chez Platynothrus peltifer (Koch) (Acarien Oribate). C R Acad Sci. 1958:247: 1655–1657.

Tucker AE, Ackerman MS, Eads BD, Xu S, Lynch M. Population-genomic insights into the evolutionary origin and fate of obligately asexualDaphnia pulex. Proc Natl Acad Sci U S A. 2013:110:15740–15745. https://doi.org/10.1073/pnas.1313388110.

Van Der Kooi CJ, Schwander T. On the fate of sexual traits under asexuality. Biol Rev Camb Philos Soc. 2014:89:805–819. https://doi.org/10.1111/brv.12078.

Vergara D, Jokela J, Lively CM. Infection dynamics in coexisting sexual and asexual host populations: support for the Red Queen hypothesis. Am Nat. 2014:184:S22–S30. https://doi.org/10.1086/676886.

Wang X, et al. The genomic selfing syndrome accompanies the evolutionary breakdown of heterostyly. Mol Biol Evol. 2020:38:168–180. https://doi.org/10.1093/molbev/msaa199.

Wang X, et al. Clonal longevity and the enigmatic flowering of woody bamboos are associated with rates of protein evolution. J Integr Plant Biol. 2025:67:2945–2963. https://doi.org/10.1111/jipb.70019.

Weber CC, Whelan S. Physicochemical amino acid properties better describe substitution rates in large populations. Mol Biol Evol. 2019:36:679–690. https://doi.org/10.1093/molbev/msz003.

Wertheim JO, Murrell B, Smith MD, Pond SLK, Scheffler K. RELAX: detecting relaxed selection in a phylogenetic framework. Mol Biol Evol. 2014:32:820–832. https://doi.org/10.1093/molbev/msu400.

West N, Lively N, Read N. A pluralist approach to sex and recombination. J Evol Biol. 1999:12:1003–1012. https://doi.org/10.1046/j.1420-9101.1999.00119.x.

Wright SI, Kalisz S, Slotte T. Evolutionary consequences of self-fertilization in plants. Proc R Soc Lond B Biol Sci. 2013:280: 20130133. https://doi.org/10.1098/rspb.2013.0133.

Wright SI, Lauga B, Charlesworth D. Rates and patterns of molecular evolution in inbred and outbred arabidopsis. Mol Biol Evol. 2002:19:1407–1420. https://doi.org/10.1093/oxfordjournals.molbev.a004204.

Xu S, Omilian AR, Cristescu ME. High rate of large-scale hemizygous deletions in asexually propagating daphnia: implications for the evolution of sex. Mol Biol Evol. 2011:28:335–342. https://doi.org/10.1093/molbev/msq199.

Yan Z, Qi G, Yao T, Li Y. Mitochondrial genomes of two asexual Trichogramma (Hymenoptera: Trichogrammatidae) strains and comparison with their sexual relatives. Insects. 2022:13:549. https://doi.org/10.3390/insects13060549.

Yang Z. PAML 4: phylogenetic analysis by maximum likelihood. Mol Biol Evol. 2007:24:1586–1591. https://doi.org/10.1093/molbev/msm088.

Yang Z, Bielawski JP. Statistical methods for detecting molecular adaptation. Trends Ecol Evol. 2000:15:496–503. https://doi.org/10.1016/s0169-5347(00)01994-7.

Yang Z, Nielsen R. Codon-substitution models for detecting molecular adaptation at individual sites along specific lineages. Mol Biol Evol. 2002:19:908–917. https://doi.org/10.1093/oxfordjournals.molbev.a004148.

Yarbrough E, Chandler C. Patterns of molecular evolution in a parthenogenic terrestrial isopod (Trichoniscus pusillus). PeerJ. 2024:12:e17780. https://doi.org/10.7717/peerj.17780.

Zhang J, Nielsen R, Yang Z. Evaluation of an improved Branch-Site likelihood method for detecting positive selection at the molecular level. Mol Biol Evol. 2005:22:2472–2479. https://doi.org/10.1093/molbev/msi237.

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