Genomic consequences of asexuality in animais

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Genomic consequences of asexuality in animals

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Prof. Ron Stoop

Abstract

Asexuality is predicted to have profound genome-wide consequences due to the absence of recombination and need of chromosomal pairing. The predicted consequences of this include: accumulation of deleterious mutations, divergence between haplotypes, changes in the dynamics of transposable elements and genomic rearrangements. Numerous case studies of individual asexual animals have tested these predictions on a genome scale, but usually only in a single asexual lineage. Several of the studied asexual genomes carried peculiar genomic features such as high rates of acquired genes via horizontal gene transfer. However, it is unclear whether the results of these studies are lineage-specific or general consequences of asexuality. In this thesis I address these gaps in three studies. i) We reanalyzed published genomes of 24 asexual animals and found that not a single genome feature is systematically replicated across a majority of these species, suggesting that there is no genomic feature characteristic of asexuality. We found that high heterozygosity levels characterized only asexuals of hybrid origin. Asexuals that were not of hybrid origin appeared to be largely homozygous, independently of the cellular mechanism underlying asexuality. ii) We sequenced genomes of five asexual *Timema* stick insects and their sexual sister species to assess the consequences of asexuality on heterozygosity, structural variations and transposable element abundance. We found convergent heterozygosity loss in all five asexual *Timema* species. We found that the homogenization mechanism applies also to structural rearrangements but to a lesser extent. iii) In a study of transposable element dynamics in experimental sexual and asexual *Saccharomyces cerevisiae* populations, we provide direct evidence that asexual reproduction drives a reduction of transposable element loads. We show, using simulations, that this reduction occurs via evolution of transposable element activity, most likely via increased excision rates. Overall, despite the importance of recombination rate variation for understanding the evolution of sexual animal genomes, the genome-wide absence of recombination does not appear to have the dramatic effects which are expected from classical theoretical models. The lack of dramatic effect of asexuality on genome evolution is surprising in the light of the dramatic consequences observed in experimental conditions. The reasons for this are probably a combination of lineage-specific patterns, impact of the origin of asexuality, and a survivor bias of asexual lineages.

Resumé français

La reproduction asexuée est souvent prédite comme une impasse évolutive. En effet, ce mode de reproduction aurait de profondes conséquences sur l'ensemble du génome, notamment en raison de l'absence de recombinaison, ou encore de l'isolement des chromosomes homologues. Ces conséquences néfastes peuvent se traduire par une accumulation de mutations délétères, une divergence entre haplotypes, un changement dans la dynamique des éléments transposables et/ou des réagencements génomiques. De nombreuses études, en général au sein d'individus d'une même lignée asexuée, ont testé ces prédictions à l'échelle du génome. Les résultats qui en découlent mettent en exergue la présence de caractéristiques génomiques propres aux asexués (par exemple : l'acquisition d'un taux élevé de gènes par transfert horizontal). Cependant, ces observations sont difficiles à interpréter car elles pourraient être spécifiques à la lignée étudiée et ne pas être liées aux conséquences générales de l'asexualité. C'est pourquoi, dans ce travail de thèse, nous avons considéré cette question par le biais de trois études distinctes : (1) Pour commencer, nous avons ré-analysé les génomes de 24 lignées asexuées différentes. Nous avons constaté qu'aucune caractéristique commune n'est répliquée au sein de leurs génomes, ce qui suggère qu'il n'existe donc pas de conséquence générale due à l'asexualité. Seuls les asexués d'origine hybride sont hautement hétérozygotes, les autres étant majoritairement homozygotes, et ce, indépendamment du mécanisme cellulaire sous-jacent. (2) Ensuite, nous avons séquencé les génomes de dix espèces de phasmes du genre *Timema*, cinq asexuées et leurs espèces sexuées les plus proches, afin d'évaluer les conséquences de l'asexualité sur l'hétérozygotie, les variations structurelles et l'abondance des éléments transposables. Nous avons constaté une perte d'hétérozygotie convergente chez les cinq espèces asexuées, et ce mécanisme d'homogénéisation s'applique également aux réarrangement structurels, mais dans une moindre mesure. (3) Enfin, grâce à une étude expérimentale sur la dynamique des éléments transposables chez *Saccharomyces cerevisiae*, nous avons apporté la preuve que la quantité d'éléments transposables est drastiquement diminuée par la reproduction asexuée. De plus, à l'aide de simulations, nous avons montré que cette réduction provient de l'évolution de l'activité de ces éléments transposables, très probablement à cause de l'augmentation des taux d'excisions.

En résumé, malgré l'importance de la recombinaison génétique dans le règne animal, phénomène maintenu par la reproduction sexuée, l'absence de cette recombinaison chez les organismes asexués ne semble pas avoir les effets dramatiques prédits par les modèles théoriques. Au vu des résultats obtenus en conditions expérimentales, l'absence d'effets néfastes de l'asexualité sur l'évolution du génome est surprenante, mais pourrait provenir d'une combinaison de raisons. En effet, chaque lignée asexuée possède des caractéristiques qui lui sont propres, une origine différente, et un biais de survie, qui pourraient expliquer ce résultat inattendu.

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Chapter 1

Introduction

Kamil S. Jaron

Introduction

Meiosis and recombination likely evolved once in the common ancestor of eukaryotes (Cavalier-smith, 2002; Ramesh, Malik, & Logsdon, 2005). Regardless if the original function was related to genetic exchange, meiosis was the key element in the evolution of true sexual reproduction - periodical cycles of genome separation (segregation and recombination during meiosis) and fusion (syngamy). Sex is a prevalent trait shared by nearly all eukaryotes, suggesting the evolutionary advantage of sex. However, what *exactly* is the significance is a matter of long discussions without any satisfactory conclusion (selected contributions to the discussion Barton & Charlesworth, 1998; Bell, 1982; Neiman, Lively, & Meirmans, 2017; Otto & Lenormand, 2002). Aside from the key features of gamete fusion and meiosis, sexual reproduction is diverse in form. In this thesis, I will focus primarily on the form of sexual reproduction found in animals for which sex is typically associated with additional features such as anisogamy and separate sexes. As this decision is pragmatic rather than biological, I will discuss the relevance to other eukaryotic taxa in Chapter 5.

Sex and recombination are prevalent, but a number of exceptions exist. Recombination suppression is often found in chromosomes carrying sex determining loci (sex chromosomes), and as a consequence non-recombining portions of sex chromosomes often degenerate (reviewed in Bachtrog, 2013). I will focus however on another exception - asexual species, species where unreduced female gametes develop without fertilization. Asexual species are rare, representing ~0.1% of species diversity and usually found on the tips of tree of life with only a few exceptional old asexual lineages (Schurko, Neiman, & Logsdon, 2009).

Asexual species provide us with a unique opportunity to understand the consequences of evolution without sexual reprodution and recombination. However, asexuals represent a diverse set of species. The predicted consequences of asexuality depend on the origin of asexuality (mutation, hybridization, ...), and the cellular mechanism of asexuality. In this chapter

first I provide an overview of the two, and then review all the predicted genomic consequences addressed in the following three chapters.

Transitions to asexuality

The loss of sex in a species is associated with two major changes in the reproduction cycle: (1) production of unreduced gametes and (2) initiation of development of embryos from unfertilized gametes (reviewed in Neiman, Sharbel, & Schwander, 2014). The production of unreduced gametes is required to generate a stable system without cumulative changes in ploidy between generations, and can be achieved by various cellular mechanisms (described in the following section). Initiation of embryonic development in sexual species is often associated with fertilization. By the definition of asexuality, embryonic development of gametes of asexual species cannot be triggered by fertilization. The possible alternative routes are spontaneous development or pseudogamy (discussed below). More than just these two traits that are associated with transitions to asexuality (e.g. behavioral changes Pijls, Steenbergen, & Alphen, 1996), however production of unreduced gametes and initiation of embryo development from unfertilized gametes represent the major obstacle as they are two large phenotypic changes that need to happen at the same time.

The transition itself then be achieved via several routes (reviewed in Neiman et al., 2014). It can be caused by **mutations** within sexual lineage (Figure 1.1A), as proposed in *Timema* stick insects (Schwander & Crespi, 2009). The origin by mutation suggests that initially the genome of the asexual species was very similar to the genome of its sexual ancestor. The second type of transition to asexuality from a single sexual ancestor is via **endosymbiont infection** (Figure 1.1B), e.g. *Wolbachia* or *Cardinium* (Stouthamer, Luck, & Hamilton, 1990; Zchori-Fein, Perlman, Kelly, Katzir, & Hunter, 2004). This transition is relatively common among haplodiploid species (van der Kooi, Matthey□Doret, & Schwander, 2017), for example in the parasitoid wasp *Leptopilina clavipes* (Pannebakker, Pijnacker, Zwaan, & Beukeboom, 2004) or thrips *Aptinothrips rufus* (van der Kooi & Schwander, 2014). Endosymbiont-

induced asexuality is very often associated with gamete duplication (reviewed in Werren, 1997), a cellular mechanism of asexuality that induces a complete loss of heterozygosity in a single generation, and therefore endosymbiont induced asexuality is expected to be associated with an immediate and complete heterozygosity loss. It is important to highlight that these endosymbionts are common among sexual species too. For example, *Wolbachia* infection is estimated to be present in around 40% of arthropod species (Zug & Hammerstein, 2012).

The third origin of asexual lineages is an **hybrid origin** (Figure 1.1C), following an hybridization event between diverged sexual populations or species, that result in asexual progeny. The exact mechanisms that lead to asexuality during hybridization are currently not clear, and possible explanations are reviewed in (Neiman et al., 2014). Asexual species of hybrid origin have been documented in various taxa, including vertebrates e.g. *Poecilia formosa* (Hubbs & Hubbs, 1932; Warren et al., 2018), *Lophotrochozoa* e.g. ribbon worms (Ament-Velásquez et al., 2016) and, with recent genomic evidence, hybrid origin seems to be frequent in nematodes e.g. *Meloidogyne* (Lunt, 2008)*, Diploscapter* (Hiraki et al., 2017) and *Panagrolaimus* (Schiffer et al., 2017). The main genomic consequence of hybrid origin is initially high heterozygosity, corresponding to the divergence of the two ancestral populations. Hybridization in general is also often associated with polyploidy (Otto, 2007), but not all asexuals of hybrid origin are polyploid (e.g. grasshopper *Warramaba virgo* is a diploid hybrid; Webb, White, Contreras, & Cheney, 1978). It has been proposed that hybrids might experience higher activity of transposable elements due to mismatch between transposable elements and silencing mechanisms. This hypothesis is further supported in a few sexual hybrid species where activation of transposable elements was observed, such as Lake whitefish (Dion-Côté, Renaut, Normandeau, & Bernatchez, 2014), Sculpin fish (Cottus) (Dennenmoser, Sedlazeck, Iwaszkiewicz, Nolte, & Altm, 2017) or Wallabies (O'Neill, O'Neill, & Graves, 1998). This hypothesis still remains to be explored in asexual species.

The three mechanisms described above are the only mechanisms that give an origin to a “de novo” asexual lineage. However, there are several documented cases of asexual species that are able to produce occasional males (e.g. the parasitoid wasp *Lysiphlebus fabarum* (Engelstädter, Sandrock, & Vorburger, 2011), and the water flea *Daphnia*; Innes, Fox, & Winsor, 2000). These males are not able to mate with obligately asexual females, but in some species they are able to mate with females of the closely related sexual species. If the male passes all necessary alleles that cause asexuality, the resulting offspring will be a new asexual lineage with the combined genome of the sexual and asexual species (Figure 1.1C). This phenomenon is known as “**contagious asexuality**” and it was described for example in *Daphnia* water flea (Xu, Innes, Lynch, & Cristescu, 2013) and in the pea aphid (Simon, Stoeckel, & Tagu, 2010). As a result, the asexual population is a mixture of asexual lineages with various genomic backgrounds recruited from the sexual sister species. The first asexual female must have been generated by an another transition route such as mutation or hybrid origin and contagious asexuality only gives rise to the subsequently derived asexual lineages.

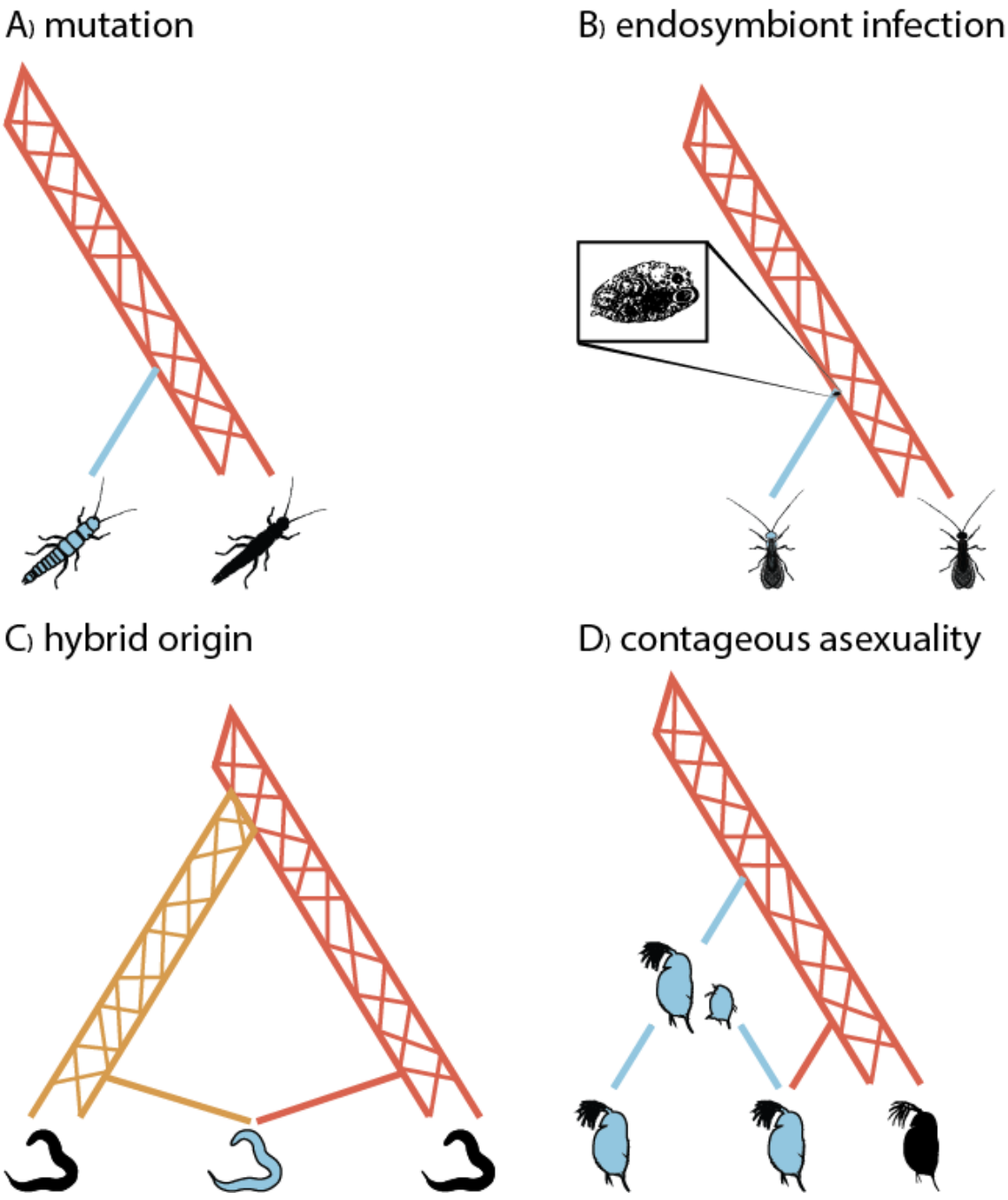

Figure 1.1: Routes of transitions from sexual to asexual reproduction. Red or yellow branches and black silhouettes represent sexually reproducing lineages, blue lines and the blue silhouettes with black contours represent asexual lineages. **A, mutation** as a cause of derived asexual lineages (e.g. *Timema*) **B, endosymbiont infection** causing asexuality (e.g. *Leptopilina clavipes* infected by *Wolbachia*) **H, hybrid origin**; hybridization between two sexual species might generate a new asexual lineage (e.g. *Meloidogyne*). **D, contagious asexuality**; accidental asexual males mating with sexual species might give rise new asexual lineages (e.g. *Daphnia*),

Cellular mechanisms of asexuality

The origin of asexuality affects the initial genomic features of the asexual species. However, the subsequent evolution is expected to be affected by the cellular mechanism of asexuality.

Cellular mechanisms of asexuality can be divided into two major categories: mitotic asexuality, also known as **apomixis**, or asexuality that involves alternated meiosis known as **automixis** (Figure 1.2, reviewed in more detail in Suomalainen, Saura, & Lokki, 1987). Females reproducing via mitotic asexuality, also known as **apomicts**, are expected to generate identical offspring with the exception of rare novel mutations. This is the most frequently considered cellular mechanism of asexuality in the theoretical studies. All the following cellular modes of reproduction involve some form of meiosis. **Endoduplication** refers to doubling of chromosomes before meiosis. During meiosis the identical duplicated chromosomes pair preferentially, therefore recombination events will not affect the genotype of unreduced gametes, which are clones of their mother. Although mechanistically it is a very different process, in practice endoduplication can be considered for many questions as “functionally mitotic”. The other extreme with respect to retention of heterozygosity is **gamete duplication**, when a normal meiosis takes place, but the haploid genome in the final gamete undergoes duplication, restoring the original ploidy levels while losing all the heterozygosity in a single generation. Gamete duplication is in most frequently found in species with endosymbiont-induced asexuality. The two intermediate meiotic cellular mechanisms involve some, but not complete, loss of heterozygosity between generations. In **automixis central fusion**, the two homologous chromosomes fuse and form an unreduced gamete. Heterozygosity is lost only if recombination occurs, and it is retained around centromeres. **Automixis terminal fusion** is almost identical to central fusion, with the difference that the chromosome duplicates fuse. In the absence of recombination, terminal fusion is equivalent to gamete duplication. Heterozygosity might be retained around telomeric regions, but only when exactly one crossover per chromosome per generation occurs during recombination.

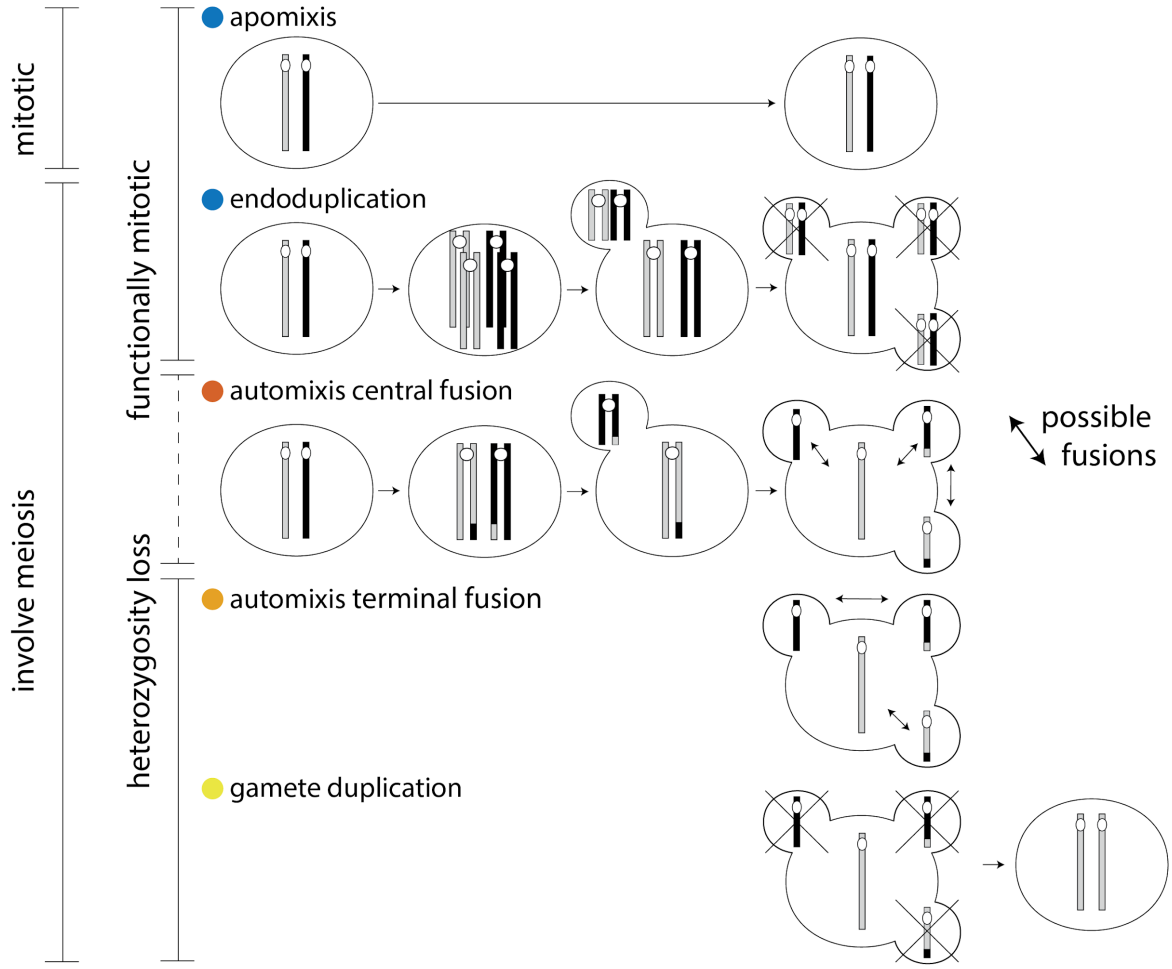

Figure 1.2: Cellular mechanisms of asexuality. **Apomixis** is asexuality via mitosis. **Endoduplication** involves meiosis, however the final gamete is an exact clone of the maternal cell and therefore endoduplication if functionally mitotic. During **automixis central fusion** gamete lose heterozygosity if recombination occurs. Although a complete recombination suppression will lead to functionally mitotic offspring (therefore the dashed line). **Automixis terminal fusion** leads to a rapid loss of heterozygosity as the fused product retain heterozygosity only when recombination is present. **Gamete duplication** leads to complete loss of heterozygosity in a single generation. The colours associated with cellular mechanisms are consistent with Chapters 2 and 5. This figure was adapted from (Neiman et al., 2014).

Gene conversion

In the absence of recombination, weaker evolutionary forces, such as gene conversion, take have significant consequences for genome evolution. Mitotic gene conversion is a mechanism similar to recombination, that leads to homogenization of heterozygous genotypes (reviewed in Chen, Cooper, Chuzhanova, Férec, & Patrinos, 2007). Gene conversion act mostly between alleles of homologous chromosomes, but has also been observed to homogenize paralogs (Jackson & Fink, 1981). Mitotic gene conversion has been shown to keep homozygous approximately 3% of regions located in a peculiar palindrome structures on human Y chromosome (Rozen et al., 2003). These regions are enriched for genes, which led to the suggestion that gene conversion might have an adaptive value for maintaining genes on the Y chromosome. The potential relevance of gene conversion was further supported by observation of gene conversion during asexual cycles of *Daphnia* water flea (Keith et al., 2016; Omilian, Cristescu, Dudycha, & Lynch, 2006). Gene conversion is also the current explanation of maintained homozygosity between homologous chromosomes in bdelloid rotifers despite of at least 40 million years of ameiotic evolution (Flot et al., 2014; Nowell et al., 2018).

Predicted genomic consequences

Many of the theoretical studies addressing the role of sex in evolution do not generate any particular predictions about genome evolution. For example studies of geographic parthenogenesis (recently reviewed by Tilquin & Kokko, 2016). Here, I focus strictly on theories that generate concrete predictions for genome evolution. In this chapter, I don’t intend to provide a comprehensive review of the empirical evidence for individual models. The examples mentioned are meant as small clues about relevance of the individual models. The extensive comparison of these models with empirical data is the main subject of the following chapters of this thesis.

Reduced efficiency of natural selection

Asexual genomes suffer a major long-term disadvantage compared to sexual, recombining, genomes: reduced efficiency of natural selection. Several models that have been proposed, such as Muller's ratchet (Muller, 1932), Ruby in Rubbish (Peck, 1994) or Hill-Robertson interference (Hill & Robertson, 1966), have been demonstrated to be caused by an identical underlying mechanism: reduced efficiency of natural selection (Felsenstein, 1974). The logic I am using to explain the effect of linkage to basics of population genetics is largely inspired by fifth chapter of the book Lost sex (Rice & Friberg, 2009).

Muller's ratchet is a principle that explains why asexual lineages suffer higher mutational load due to accumulation of deleterious mutations. For illustration, imagine a population of individuals grouped into fitness classes. In a finite population, due to stochastic processes it might happen that all the individuals of the most fit genotype in the population mutate or are lost by genetic drift. In a sexual population the very same high fitness class can be restored by recombination and segregation of alleles. In contrast, the asexual population has lost the most fit genotype forever - the ratchet irreversibly clicks by one tooth to a higher mutational load. This process can be view as an analogy of fixation of deleterious variants in sexual species due to genetic drift, however the theory predicts that the mutation load of asexual populations is substantially higher compared to otherwise identical sexual species (Kimura & Maruyama, 1966; Muller, 1932).

In the absence of recombination, every mutation, whether advantageous or deleterious, is trapped in the genetic background it appeared in. The linkage of variants often generates interference in responses to selective forces, thereby reducing the efficiency of selection (Hill & Robertson, 1966). This process is called Hill-Robertson interference and as mentioned earlier, it is caused by the very same mechanism as Muller's ratchet, although the formulation of the problem is very different. The second consequence of mutations being trapped in their genetic background is competition between

beneficial mutations that occurred in different individuals, as these mutations will never meet. The only way for asexual population to adapt is by a series of consecutive beneficial mutations in the same lineage, which is a far more improbable process compared to adaptation in sexual populations of same size.

Muller's ratchet was described for haploid genomes, but the logic should apply for higher ploidy levels as long as the inheritance is clonal. However the assumption of clonality is very frequently violated, either by mitotic gene conversion, or even recombination in asexual species reproducing via one of the meiotic cellular mechanisms. Both gene conversion and meiosis generate higher variability among offspring compared to strictly clonal reproduction. In the context of sex chromosome evolution, it was shown that gene conversion might slow down or completely reverse the effects of Muller's ratchet, if the rates of gene conversion are high enough (Marais, Campos, Gordo, & Lyon, 2010). Presumably the same could be true for meiotic asexuals, however this has never been shown quantitatively.

Prediction: Asexual species are expected to suffer a **higher mutational load** and display **weaker signatures of positive selection** compared to sexual species.

In Chapter 2 evidence regarding this prediction is reviewed and discussed.

Intragenomic variability

The intragenomic divergence of asexuals that reproduce via mitotic asexuality or endoduplication is expected to exceed the intergenomic divergence in the population (Birky, 1996), this model is known as "Meselson effect" after one of the authors of the first report in bdelloid rotifers (Welch, D. M., Meselson M, 2000). Ironically, these authors later showed that the evidence for a Meselson effect was misinterpreted due to degenerated tetraploid genome of bdelloid rotifers (Welch, Welch, & Meselson, 2008). The Meselson effect predicts that the phylogeny of phased haplotypes of an old apomictic diploid population

should show two branches with identical topologies, representing phylogenies of the two haplotypes (Figure 1.3). As a consequence, intragenomic diversity is predicted to increase over time in apomictic species. The accumulation of intragenomic diversity can, however, be counteracted by mitotic gene conversion. The expected intragenomic diversity then depends on the relative frequency of gene conversion versus mutation. It has been shown in the water flea *Daphnia* that gene conversion homogenize the genome faster than mutations occur (Omilian et al., 2006). The full extent of the role of gene conversion for intragenomic divergence remains to be explored.
Meiotic asexual species are expected to lose heterozygosity over time. The speed depends on the cellular mechanism and the recombination rate. Species reproducing via automixis central fusion might be able to retain heterozygosity around centromeres. Species with terminal fusion are expected to lose heterozygosity very quickly. The only mechanism that allows a retention of heterozygosity in automixis terminal fusion is a stable, odd number of recombination events as is the case, for instance, in the sexual species *Caenorhabditis elegans* (Hillers & Villeneuve, 2003). The retained heterozygosity is then expected to be located near telomeres. Species reproducing via gamete duplication are not expected to carry any intragenomic divergence at all as the genome is fully homogenized every generation.

Prediction: **Intragenomic diversity** is expected to **increase over time** in apomictic species, but not other asexuals.

This prediction is not directly tested, but is discussed at length in Chapter 5, using results from Chapter 2.

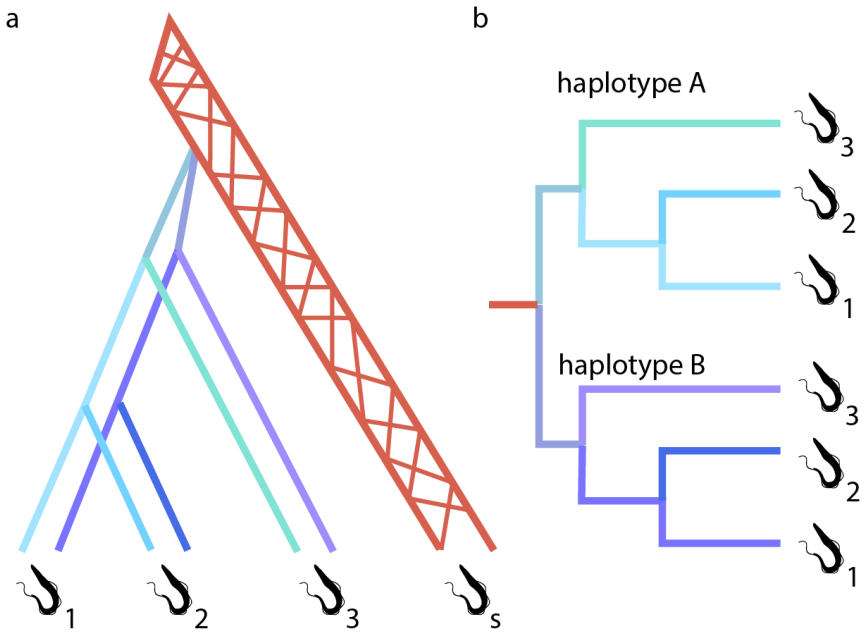

Figure 1.3: “Meselson effect” generated by independent mutation accumulation in the two haplotypes. **a** An asexual lineage with a single origin derived from a sexual ancestor plotted as an overlapping cladogram. As the haplotypes slowly diverge, their common ancestor is at the very origin of asexuality. However the common ancestor of haplotypes of other individuals dates to their common asexual ancestor. **b** The identical cladogram shown witout overlap of branches corresponding to haplotypes in a single individual.

Structural variations

The divergence between haplotypes can happen both at the nucleotide sequence level, and at the level of rearrangements. Genome rearrangements result in a few types of possible structural variations, insertions, deletions, duplications, inversions or translocations. In sexual species structural variations are rarer than single nucleotide polymorphisms, but account for major phenotypic changes (Jeffares, 2016; Joron et al., 2011; Sudmant, Alexis, & Burge, 2015). Structural variations, inversions in particular, are also associated with obstruction to recombination (Stevison, Hoehn, & Noor, 2011), which might counteract present structural variations in sexual species. Apomictic asexuals are supposedly free from this constraint, and are therefore expected to harbour more structural variations compared to sexual sister species. Overall genetic variability might be different between sexual and asexual species, and therefore we formulate the hypothesis relative to the rest

of the genetic variability. We hypothesise that the fraction of genetic variation carried by structural variants is higher in apomictic asexuals than in sexual species. Furthermore, obstructions to recombination might not be strongly selected against even in automictic species as the potential benefit of recombination is smaller (if any).

Prediction: Asexual species are expected to carry a **higher proportion** of **genetic variability** by **structural variations** compared to sexual species.

This prediction is addressed in Chapter 3 in five asexual *Timema* stick insects.

Intergenomic variability

In sexual species genetic diversity is generated by segregation, recombination and mutations. In asexual species the diversity is generated only via mutations. Furthermore positive selection is expected to lead to a greater decline of genetic variability in asexual species as the whole genome hitchhike with the selected locus (Fisher, 1930). The intergenomic (population) variability in therefore expected to be smaller in asexual populations compared to sexual species.

These thoughts are based simply on principles of population genetics, considering variant with exactly same fitness effects in sexual and asexual species, which is presumably true for single nucleotide polymorphisms. However, larger genomic rearrangements (structural variations) can obstruct recombination (Stevison et al., 2011) and therefore might be deleterious in sexual species, while being nearly neutral in asexual species.

Transposable element dynamics

In sexual species, selfish copy-paste transposition increases the transmission rate of the transposable element at the expense of the fitness of the whole genome. Transposition process in an asexual genome will, however, not affect the transmission rate as the transposon cannot colonize new genomic

backgrounds Hence in an asexual genome a transposition event acts as a mutation. Due to a lower efficiency of selection in asexual species, transposable elements might thus increase in the short term after the transition to asexuality. The transposons are expected to evolve benign over time in asexuals (Charlesworth & Langley, 1986). The accumulation or reduction of transposons in an asexual genome depends on the population size, and the transposition and excision rates of transposable elements (Dolgin & Charlesworth, 2006). The model of Dolgin & Charlesworth (2006) assumes a finite haploid apomictic population derived from an infinite sexual population at transposition-excision-selection equilibria.

Prediction: **Transposable element load is changed** after a transition to **asexuality**.

In Chapter 2 we estimate transposable element load in 24 asexual species, but we lack a comparison to their sexual sister species. In Chapter 3 we compare transposable element load in five asexual species of *Timema* stick insects and the loads of their sexual sister species. In Chapter 4 we use a yeast experiment to access transposable element dynamics in a controlled environment, and further expand the model of Dolgin & Charlesworth (2006) to relax the assumptions not matching the yeast experimental design.

Concluding remarks and authors contributions

Here I have described possible origins of asexuality and the cellular mechanisms maintaining them. In the second part of the introduction I have reviewed some predicted genomic consequences of asexuality that will be compared to empirical data in the following chapters. The following chapters focus on genomic consequences observed in nature or in experimental settings. In the second chapter we generate a catalogue of genomic features of 24 asexual animals, and thereby define the ranges of genomic properties so far observed in asexual animals. I gathered, analyzed and visualized the data with input from all co-authors. The manuscript was drafted by me and Jens Bast with a lot of input also from Marc Robinson-Rechavi and Tanja

Schwander. The third chapter addresses the direct consequences of asexuality in a genus of Californian stick insects *Timema,* by comparing the asexual lineages to their sexual sister species. I was responsible for processing the raw sequencing data, genome assembly, and all the analyses presented here except of the analysis of transposable elements. The fourth chapter explores the interplay between sexual reproduction and transposable element dynamics in an experimental evolution study using yeast. I was responsible for the theoretical part of the study, with input from Jens Bast, Denis Rose and Tanja Schwader. In chapter 5 I discuss the generality of the genomic consequences, both in animals but also with regard to other eukaryotic taxa, and further propose future directions for studies of the consequences of asexuality.

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Chapter 2

Genomic features of asexual animals

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Chapter 2: Genomic features of asexual animals

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Data Availability: The code of the pipeline for gathering data, calculating and plotting results is available at https://github.com/KamilSJaron/genomic-features-of-asexual-animals; the majority of sequencing reads are available in public databases under the accessions listed in **Supplementary Table 1**. The data without publicly available sequencing reads were obtained via personal communication with the corresponding authors.

Abbreviations: TE, transposable element; HGT, horizontal gene transfer

Abstract

Evolution under asexuality is predicted to impact genomes in numerous ways, but empirical evidence remains unclear. Case studies of individual asexual animals have reported peculiar genomic features which were suggested to be caused by asexuality, including high heterozygosity, a high abundance of horizontally acquired genes, a low transposable element load, and the presence of palindromes. We systematically characterized these genomic features in published genomes of 26 asexual animals representing at least 18 independent transitions to asexuality. Surprisingly, not a single feature is systematically replicated across a majority of these transitions, suggesting that no genomic feature is characteristic of asexuality and that previously reported patterns were lineage specific rather than caused by asexuality. We found that only asexuals of hybrid origin were characterized by high heterozygosity levels. Asexuals that were not of hybrid origin appeared to be largely homozygous, independently of the cellular mechanism underlying asexuality. Overall, despite the importance of recombination rate variation for understanding the evolution of sexual animal genomes, the genome-wide absence of recombination does not appear to have the dramatic effects which are expected from classical theoretical models. The reasons for this are probably a combination of lineage-specific patterns, impact of the origin of asexuality, and a survivor bias of asexual lineages.

Introduction

Sex: What is it good for? The reason why most eukaryotes take a complicated detour to reproduction, when straightforward options are available, remains a

central and largely unanswered question in evolutionary biology [1,2]. The species in which asexual reproduction is the sole form of replication typically occur at the tips of phylogenies and only few of them have succeeded as well as their sexually reproducing counterparts [3]. In other words, most asexual lineages may eventually be destined for extinction. These incipient evolutionary failures, however, are invaluable because, by understanding the evolutionary fate of asexual species, something may be learned about the adaptive value of sex.

An increasing number of studies have sequenced the genomes of individual asexually reproducing animals, often with the aim of identifying features that distinguish them from sexual species (**Figure 1**). In asexual animals, females produce daughters from unfertilized eggs via so-called thelytokous parthenogenesis (hereafter asexuality) [4]. Asexuality is predicted to have many consequences for genome evolution, since gamete production via meiosis and the restoration of somatic ploidy levels via fertilization no longer take place. Predicted consequences include, for example, the accumulation of deleterious mutations [5–7], as well as changes in intragenomic heterozygosity levels [8,9] and transposable element (TE) dynamics [10]. In the present study, we evaluate whether asexual reproduction indeed generates these predicted genomic signatures by reanalyzing and comparing the published genomes of 26 asexual animal species (**Figure 1**). Previous genome studies were unable to address the general question because they focused on individual asexual lineages. Because asexuality is a lineage-level trait, disentangling causes of asexuality from lineage-level characteristics

requires replication across independently evolved instances of asexuality. Our study includes species from at least 18 independently evolved asexual lineages, providing us with the unique opportunity to detect universal consequences of asexuality that are not confounded by lineage-specific patterns. Furthermore, we study the same features in all genomes, whereas different genome studies focused on different genomic features (Figure 1), which thus far precluded broad comparisons across different asexual groups. Finally, our dataset includes four species of bdelloid rotifers, a group that likely persisted and diversified in the absence of canonical sex for over 40 million years [12]. Bdelloids have thus far overcome the predicted dead-end fate of asexuality, which raises the question of what mechanisms protect them from extinction, and whether these mechanisms are visible in specific characteristics of their genomes.

Because the predicted consequences of asexuality are strongly affected by how asexuality evolved from the sexual ancestor (**Box 1**) as well as by the cellular mechanisms underlying asexuality (**Box 2**), we include biological differences among asexual species in our comparisons. For example, some asexual species have evolved via hybridization (**Box 1**), which generates incipient asexuals with high intragenomic heterozygosity and can result in increased activity of transposable elements [13–15]. In such instances, it can be difficult to disentangle consequences of hybridization from those of asexuality. Similarly, some cellular mechanisms underlying asexuality involve meiotic divisions, with a secondary restoration of somatic ploidy levels, while others do not. In the former case, heterozygosity in the asexual species is

expected to decay rapidly, while in the latter case, it could be maintained or even increase over time (Engelstädter 2017). Finally, because the genome studies differed in their focus and in the methods used, we reanalyzed the published genomes with standardized approaches. Whenever possible, we conducted quantitative comparisons between groups of asexual species. However, for interpretation, it is important to consider that the available genomes are neither a random nor a representative sample of asexual animals.

We uncovered a number of unusual features in the genomes of asexual animals that were not reported in the original genome studies, including extreme loads of transposable elements and asymmetric divergence among haplotypes in polyploid species of hybrid origin. However, none of these were systematically replicated across even a majority of analyzed species, let alone all of them, suggesting that there is no universal genomic feature specific to asexual species. Unexpectedly, we found that the cellular mechanism underlying asexuality has little or no impact on intragenomic heterozygosity. Asexual species of hybrid origin are highly heterozygous even when their cellular mechanism underlying asexuality should generate homozygous genomes. Asexuals that are not of hybrid origin are largely homozygous. We argue that lineage-specific patterns as well as a survivor bias of asexual lineages are the main drivers of genome features in the studied asexual animals.

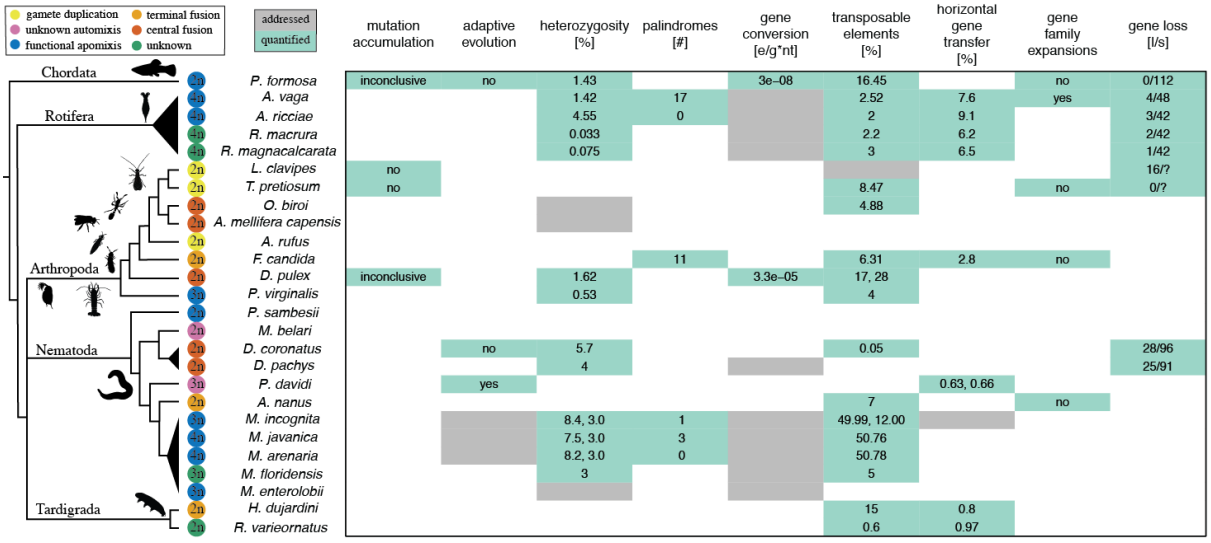


Figure 1: **Genome features studied in asexual animal species.** The phylogeny displays the taxonomic relationships of the 26 sequenced asexual animal species considered here, representing at least 18 independent transitions to asexuality. Species that might derive from the same original transition are grouped in triangles. The color of the circle indicates the cellular mechanism of asexuality and the number inside the circle the ploidy of the species (see Supplemental Table 1 for details). We note *M. floridensis* as triploid, as shown by our analyses, even though it is reported as diploid in the original paper; see Supplementary Materials S1 for details (Ranallo-Benavidez et al. 2019). Each original genome paper explored a given set of genome features: the green cells represent cases where the genomic feature was quantified (values are indicated); the grey cells represent studies where the genomic features were addressed with respect to asexuality, but the results we quantitatively incomparable to other studies. Heterozygosity, palindromes, transposable elements, and horizontal gene transfer were reanalysed in this study; the discussion of the remaining features is based on the analyses reported in the individual genome studies [16–38]. Findings for

mutation accumulation and adaptive evolution refer to comparisons between sexual and asexual species and are reported with respect to theoretical predictions (yes: as predicted, no: opposite to predictions, inconclusive: no difference). e/g*nt: **e**vent per **g**eneration per **n**ucleo**t**ide; l/s: number of **l**ost genes among the **s**tudied genes related to sexual reproduction.

Box 1: Transitions to asexuality

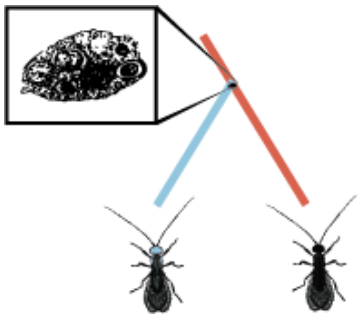
Meiotic sex and recombination evolved once in the common ancestor of eukaryotes (Cavalier-Smith 2002). Asexual animals therefore derive from a sexual ancestor, but how transitions from sexual to asexual reproduction occur can vary and have different expected genomic consequences of asexuality [14].

Hybrid origin:

Hybridization between sexual species can generate hybrid females that reproduce asexually [14,40]. Asexuality caused by hybridization generates a highly heterozygous genome, depending on the divergence between the parental sexual species prior to hybridization. Hybridization can also result in a burst of transposable element activity [13].

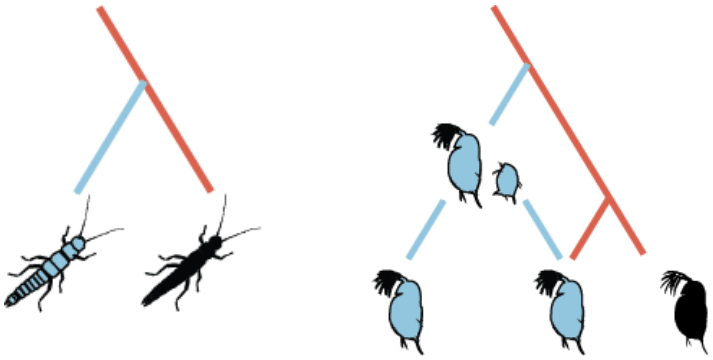
Intraspecific origins:

Endosymbiont infection. Infection with intracellular endosymbionts (such as *Wolbachia*, *Cardinium* or *Rickettsia*) can cause asexuality, a pattern that is frequent in species with haplodiploid sex determination [41]. This type of transition often (but not always) results in fully homozygous lineages because induction of asexuality frequently occurs via gamete duplication (see Box 2).



Spontaneous mutations/Contagious asexuality. Spontaneous mutations can also underlie transitions from sexual to asexual reproduction. In addition, asexual females of some species produce males that mate with females of sexual lineages, and thereby generate new asexual strains (contagious asexuality). In both cases, the genomes of incipient asexual lineages are expected to be very similar to those of

their sexual relatives and subsequent changes should be largely driven by the cellular mechanism underlying asexuality (**Box 2**).



Box 2: Cellular mechanisms of asexuality

In sexual species offspring are generated through the fusion of male and female gametes. In asexuals, females generate diploid (or polyploid) offspring from unfertilized oocytes via different cellular mechanisms. The mechanism is predicted to affect genome evolution and especially heterozygosity levels. For details see [4,42].

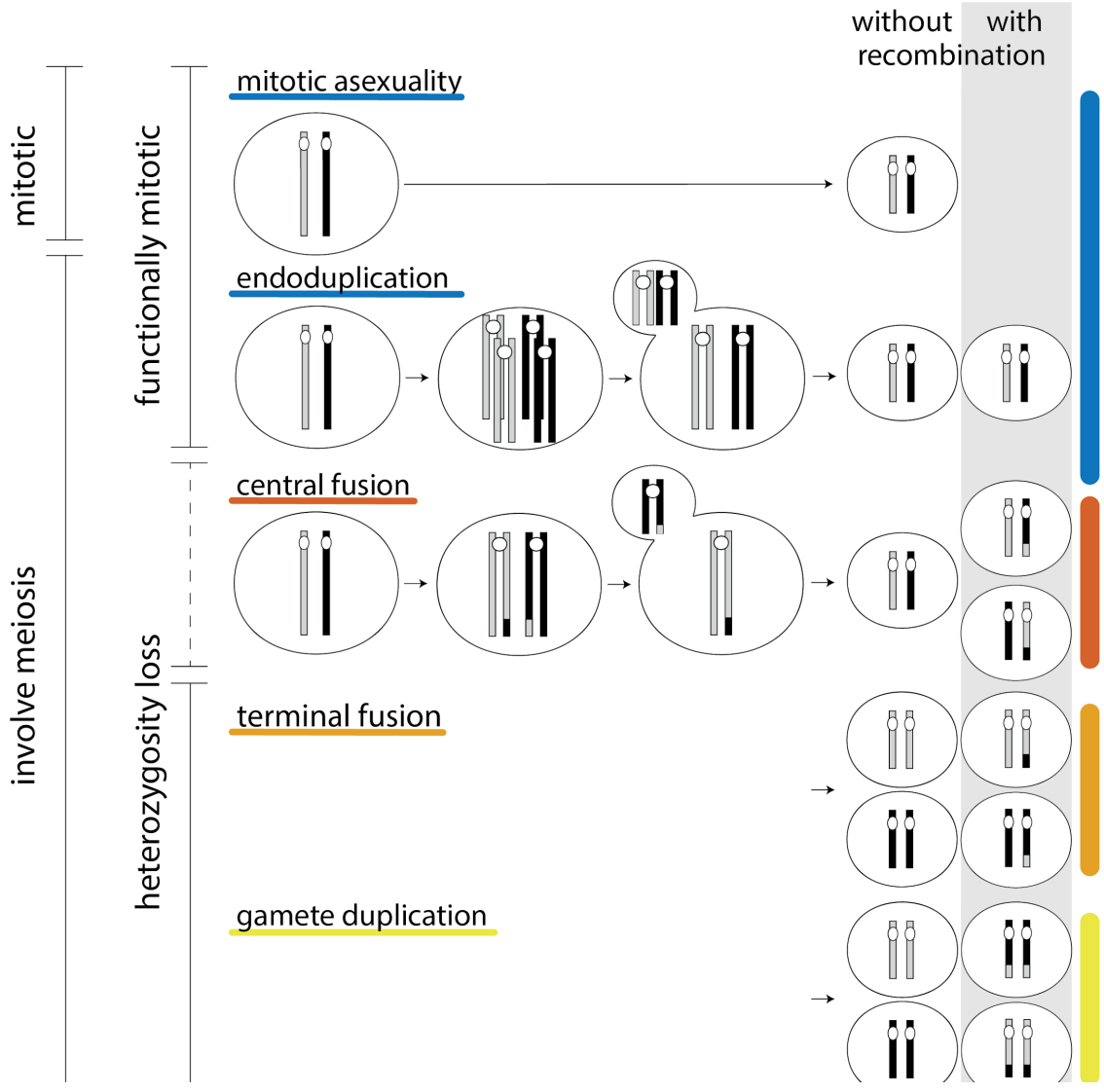
Mitotic asexuality (Apomixis). Under mitotic asexuality, no ploidy reduction occurs and offspring are clones of their mother.

Meiotic asexuality (Automixis). Under meiotic asexuality, meiotic divisions occur partially or completely, but somatic ploidy levels are maintained via different mechanisms. Some of these mechanisms have similar genomic consequences as mitotic asexuality, even though meiosis is involved (for example, endoduplication in hybrid asexuals results in offspring that are clones of their mother). Such mechanisms are often referred to as "functionally mitotic" (or functionally apomictic), especially when the cellular mechanisms are not known in detail but genotyping data suggest that offspring are clones of their mother.

> ***Endoduplication***. A duplication of the entire chromosome set occurs before normal meiosis, during which ploidy is reduced again. If recombination occurs between identical chromosome copies rather than between chromosome homologs, endoduplication produces offspring that are clones of their mother.
>
> ***Central fusion*** **and** ***terminal fusion***. Under these two mechanisms, somatic ploidy levels are restored through the fusion of two of the four meiotic products (products separated during the first meiotic division

merge under central fusion, products separated during the second division merge under terminal fusion). In the absence of recombination, central fusion generates offspring that are clones of their mother. The consequences for heterozygosity are opposite under inverted meiosis, where chromatids are separated during meiosis I and chromosomes during meiosis II. For example, terminal fusion with an inverted sequence of meiosis and no recombination (not shown here) generates offspring that are clones of their mother (see Lenormand et al. 2016 for a recent review).

Gamete duplication. After a full meiosis, a haploid meiotic product undergoes duplication. This results in a diploid, but fully homozygous offspring.



Results and discussion

We studied nine genomic features that were proposed to be affected by asexuality (**Figure 1**). Four of them represent classical theoretical predictions for consequences of asexuality on genome evolution (mutation accumulation, positive selection, transposable elements, and intragenomic heterozygosity). The five remaining ones are unusual genomic features that were observed in individual asexual species and suggested to be linked to asexuality (horizontal gene transfers, palindromes, gene conversion, gene family expansions, and gene losses). We quantified the four features (heterozygosity, palindromes, transposable elements, and horizontal gene transfer) for which the relevant genomic data are available for all or most sequenced asexual species. The remaining five genomic features require data that are not available for the majority of the asexual species. Specifically, the theoretical predictions pertaining to mutation accumulation, positive selection, gene family expansions, and gene loss are always relative to sexual species (e.g., selection is less effective in asexual than sexual species, leading to increased rates of deleterious mutation accumulation and reduced rates and strengths of positive selection) and cannot be independently quantified in asexuals. For these four genome features we synthesize the available information from the literature, including studies that are not based on whole-genome data.

Overview of species and genomes studied

We reanalyzed the published genomes of 26 asexual animal species with the aim of identifying general genomic signatures of asexualilty. The 26 species correspond to at least 18 independent transitions to asexuality and cover a

broad taxonomic range, including chordates, rotifers, arthropods, nematodes, and tardigrades. In addition to covering this taxonomic range, these asexual species vary in the cellular mechanisms underlying asexuality, in the mechanisms that caused the transition to asexuality, as well as in other biological aspects (**Figure 1, Supplementary Tables 1 & 2**). This variation allows us to assess whether asexuality generates universal genomic signatures independently of species-specific traits.

The cellular mechanisms underlying asexuality have been reported in 22 of the 26 species. Eight of them involve mitotic asexuality, while the 14 remaining species have different types of meiotic asexuality (**Figure 1**). All but one of the eight species with mitotic asexuality are polyploid, the amazon molly being the only diploid studied. Conversely, all but one species with meiotic asexuality are diploid. This is expected given that polyploidy can generate problems during meiosis (reviewed in [45]). Nevertheless, the nematode *Panagrolaimus davidi* is characterized by both meiotic asexuality and triploidy [30] (see **Supplementary Table 1** for details).

Information on how asexuality evolved is available for 16 of the 26 sequenced species (**Supplementary Table 1**). A hybrid origin has been suggested for ten of these, based on identification of parental species. Endosymbionts are the most likely cause of asexuality in four species (the springtail, both wasps, and the thrips), and spontaneous mutation in two (the ant and the cape honey bee). Across the 26 species, a hybrid origin is correlated with polyploidy. Six of the 11 polyploids in our sample are of hybrid origin, while for the five others

a hybrid origin is supported by our results (see below), even though it was not suggested previously. It is important to note however that many polyploid asexual animals are not of hybrid origin, including several well studied asexual species such as the New Zealand mudsnail *Potamopyrgus antipodarum*, the bush cricket *Saga pedo*, or the bagworm moth *Dahlica triquetrella*. None of these has a published genome yet, which precludes their inclusion in our study.

Most if not all predicted consequences of asexuality are expected to accumulate over time, meaning that their effect size as well as the power to detect them is increased in old asexual lineages. However, estimating the age of asexual lineages is difficult and always associated with large uncertainties [46,47]**.** We therefore did not include quantitative comparisons among asexuals with respect to their age. However, because our set of species comprises asexuals believed to be 'ancient' (i.e., several million years old, see **Supplementary Table 1**), we discuss, where appropriate, potential age effects in a qualitative manner.

Mutation accumulation and positive selection

One of the classical predictions linked to asexuality is that it reduces the efficacy of selection [5–7,48–50]. This reduction occurs because linkage among loci in asexual species prevents selection from acting individually on each locus. This can allow deleterious mutations to accumulate over time, because they are linked to other sites under selection. It can also reduce the rate of adaptation, because beneficial mutations cannot reach fixation in a

population as easily as under sexual reproduction.

Analyzing mutation accumulation and positive selection requires comparisons of homologous gene sets in sexual and asexual relatives, which are only available in four of the 26 asexual species (Figure 1). It was therefore not possible to analyze these genome features in the present study. However, the prediction that deleterious mutations accumulate more rapidly in asexual than sexual lineages has been tested in over twenty different groups of asexual species (reviewed in [51], plus three additional studies published since [16,21,52]), with results generally supporting the prediction. However, in only eight studies were the tests conducted genome wide, while tests in the remaining studies were based on only one or a few genes. Note that four [11,52–54] of these studies were based on transcriptomes and are therefore not included in our systematic reanalysis. Among the genome-wide tests, results are much more mixed than among the studies on few genes, raising the question whether the latter are representative of the genome as a whole. Specifically, only two of the eight genome-wide studies support deleterious mutation accumulation in asexuals [52,53]. Moreover, two of the other studies found that *sexual* taxa experienced more deleterious mutation accumulation than asexual taxa [11,19], while the four remaining ones found no differences between sexual and asexual taxa [16,21,24,54]. In the case of the water flea *D. pulex*, the study specifically reported that earlier inferences of deleterious mutation accumulation under asexuality were incorrect, as the deleterious mutations detected in asexual strains were inherited from the sexual ancestor

and not accumulated after the transition to asexuality [24].

In summary, results from genome-wide studies addressing the prediction of deleterious mutation accumulation in asexual species are equivocal. More studies are therefore needed. A major constraint for studying deleterious mutation accumulation, and the reason why it was not studied in most genome studies of asexuals species (**Figure 1**), is that it requires sexual outgroups for comparison. These species are either unknown or not included in most published genome studies of asexuals.

The same constraints likely explain why no study has thus far directly addressed adaptive evolution in the genome of an asexual species. The question of adaptive evolution was addressed indirectly in the amazon molly, by studying the amount of segregating variation at immune genes (where variation is known to be beneficial). The authors found very high diversities at immune genes [16]. However, these were difficult to interpret because standing variation was not compared to that in sexual relatives, and because the amazon molly is a hybrid species. Hence the high diversity could be a consequence of the hybrid origin rather than of asexuality.

Heterozygosity

Intragenomic (individual-level) heterozygosity is the nucleotidic divergence between the haploid genome copies of an individual. In a panmictic sexual population, intragenomic heterozygosity corresponds to the genetic diversity in a population (the amount of variation observed between DNA sequences

from different individuals). This is however not the case in asexual populations, which are, by definition, not panmictic. Heterozygosity in asexuals is therefore a property of individuals, not populations.

Intragenomic heterozygosity in asexual organisms is expected to depend on three major factors: (1) the mechanism of transition to asexuality (which determines the initial level of heterozygosity; **Box 1)**, (2) the cellular mechanism underlying asexuality (which determines whether heterozygosity will increase or decrease over time; **Box 2**), and (3) how long a species has been reproducing asexually (because the effect of asexuality accumulates over time).

In diploid species, genome-wide heterozygosity can correspond to the divergence between alleles (homolog heterozygosity), or if the species has a history of hybridization, to the divergence of gene copies derived from different species (hereafter homoeologs, following the terminology of Glover et al [57]). In polyploid species, heterozygosity can be a combination of homolog and homoeolog divergence.

To compare intragenomic heterozygosity among species with different ploidy levels we estimate heterozygosity as the proportion of sites with more than one allele present among all homologous genome regions (consistent with (Lokki 1976)). For the 26 asexual genomes in our study, we distinguish homolog and homoeolog heterozygosity whenever possible, or infer a "composite heterozygosity" (the sum of the two) when the distinction is not

possible. To avoid biases stemming from variable genome assembly qualities, we estimated heterozygosity directly from sequencing reads using kmer spectra analysis (Ranallo-Benavidez et al. 2019), except for bdelloid rotifers where the heterozygosity levels exceeded the range quantifiable by this method (See **Supplementary materials S4**).

Species with an intraspecific origin of asexuality show low heterozygosity levels (0.03% - 0.83%), while all of the asexual species with a known hybrid origin display high heterozygosity levels (1.73% - 8.5%, **Figure 2**). Although the elevated heterozygosity levels are intuitively expected in species of hybrid origin, it is surprising that the pattern holds regardless of the cellular mode of asexuality.

The heterozygosity levels present at the inception of asexuality should decay over time for most forms of meiotic asexuality [42,55]. Under mitotic asexuality, heterozygosity is expected to increase over time as haplotypes can accumulate mutations independently of each other (generating the so-called 'Meselson effect') [8]. However, gene conversion can strongly reduce haplotype divergence and, if high enough, can even result in a net loss of heterozygosity over time, even under mitotic asexuality [8,17]. In spite of the prediction that the cellular mechanism of asexuality should affect heterozygosity, it appears to have no detectable effect on heterozygosity levels once we control for the effect of hybrid origins (**Figure 2**). However, we have very little power to detect such effects, especially because our dataset does not include any asexual species that uses mitotic asexuality but is not of

hybrid origin. It is interesting to note that species with different forms of meiotic asexuality (including gamete duplication and central fusion) have similarly low heterozygosity levels. This suggests that although the rate of heterozygosity loss is expected to vary according to mechanisms of asexuality, this variation is only relevant very recently after transitions to asexuality, and no longer affects heterozygosity among established asexual species. Alternatively, variation in heterozygosity caused by different forms of meiotic asexuality may be too small to be detected with our methods.

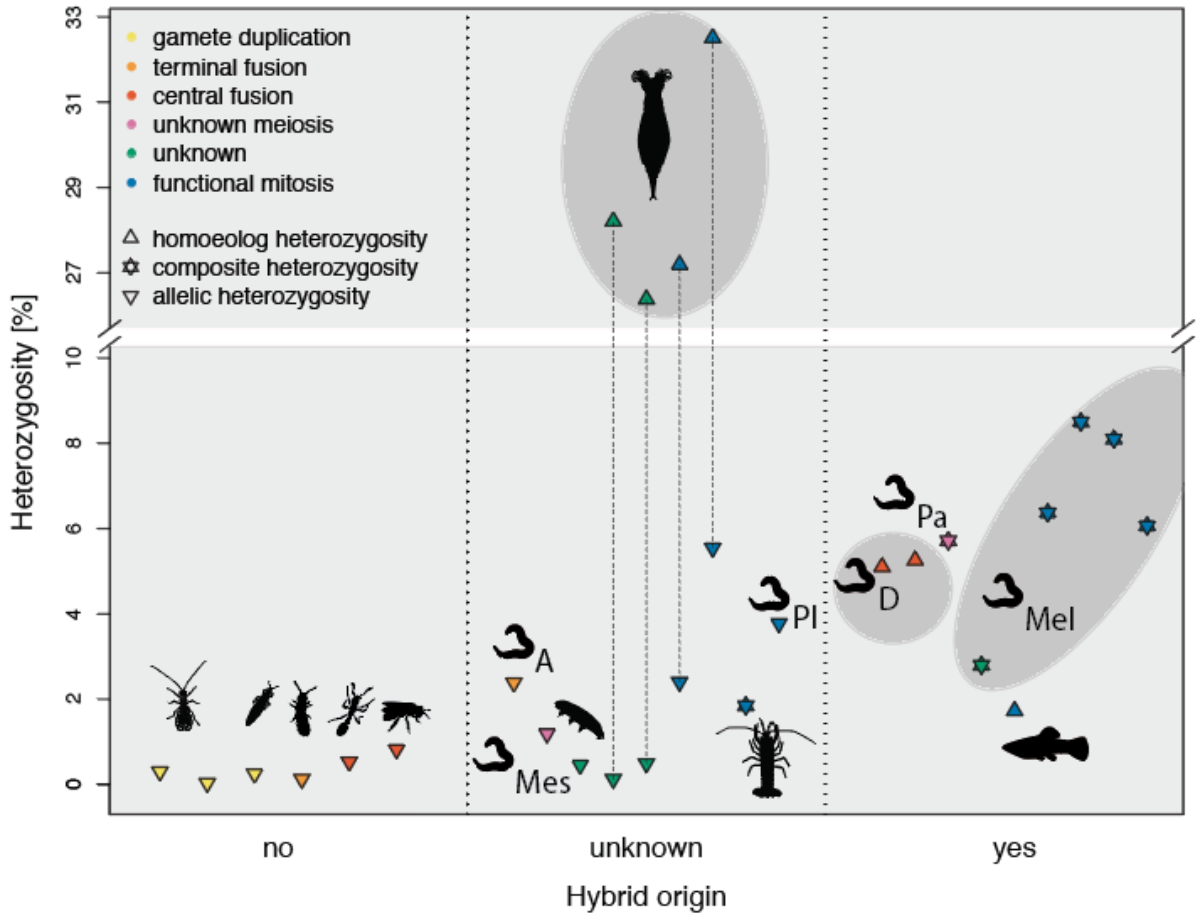


Figure 2: **Hybrid origin is the main driver of high heterozygosity in asexual species**. Heterozygosity estimates with respect to hybrid origin (x axis) and cellular mechanism of asexuality (color code). Whenever possible, heterozygosity is decomposed into heterozygosity between alleles of intra-

specific origin versus heterozygosity between homoeologs of hybrid origin (shapes). Composite heterozygosity covers the cases where the two cannot be distinguished. Functional mitosis refers to cellular mechanisms that are expected to maintain heterozygosity across generations (e.g., mitosis or endoduplication; **Box 2**). Species with a possible shared origin of asexuality are grouped in gray ellipses. Nematode genus abbreviations: Pl: *Plectus*, Mes: *Mesorhabditis*, D: *Diploscapter,* Pa: *Panagrolaimus*, A: *Acrobeloides*, Mel: *Meloidogyne*. We were unable to generate heterozygosity estimates for two of the 26 asexual species for different reasons: in the tardigrade *H. dujardini* because of extensive contamination in the sequencing reads, and in the water flea *Daphnia pulex* samples because of too low coverage (see **Methods**).

Heterozygosity structure in polyploids

Heterozygosity is estimated as the proportion of sites that differ in at least one of the homologous regions (see above). This means that in polyploids the estimated genome-wide heterozygosity could be generated by a single haplotype that is highly divergent while others are similar, or by homogeneous divergence across all copies present, or a combination of these. With the exception of bdelloid rotifers, we are not able to directly compare the divergence of individual haplotypes (because such a comparison requires phased genomes, which are available for rotifers only). However, we are able to measure the haplotype structure on a per-locus basis using k-mer based approaches implemented in GenomeScope 2.0 (Ranallo-Benavidez et al. 2019). These approaches notably allow us to distinguish biallelic from triallelic loci in triploid organisms.

The heterozygosity of two of the five triploid species in our dataset (the crayfish and nematode *M. floridensis*) are composed mostly from biallelic loci, while the remaining triploid species, show a relatively higher fraction of triallelic heterozygosity (**Figure 3**). The low proportion triallelic loci suggest AAB structure of the two genomes, where the two haploid genome copies (A) are nearly identical and the last genome copy (B) is the carrier of the observed heterozygosity. This AAB model is in agreement with the previous genomic analysis of the crayfish data. However, we report that the divergence of the third genome copy (B) exceeds by far the heterozygosity observed in the sexual sister species *P. fallax* (1.8% in *P. viginalis* compared to XXX% in *P. fallax*), we therefore suggest a hybrid origin of the B genome copy (Supplementary text S6). The root knot nematode *M. floridensis* features rather different genome structure to the other triploid *Meloidogyne* genome, this is perhaps the reason why the genome was previously mistaken for diploid (Supplementary material S1), but also suggest that the origin of triploidy in *M. floridensis* is independent of the origin of triploid in the other species in the genera. Heterozygosity in the three species with high fraction of triallelic loci is distributed more among genomic copies., but as the measure is relative, we can not possibly infer how close to equidistant the genome are. This analysis holds even if we correct for total heterozygosity observed in the genome (Supplementary material S7).

In the tetraploid species, the biallelic loci can be sorted to one divergent genome copy (yellow portions) and two genomic copies carrying the

alternative allele (pink portions). The genomes of the two tetraploid *Meloidogyne species* contain high portions of all heterozygosity structures (**Figure 3**) suggesting a complex genomic structure such as AABC and ABCD. Alternatively, this signal can be also caused by partial aneuploidy that is common in the *Meloidogyne* species.

Haplotype divergences can be used to infer the origin of asexuality in polyploid species: in asexual polyploids of hybrid origin we expect and observe highly heterogeneous divergences among haplotypes, while polyploidy of intra-specific origin is predicted to generate homogeneous divergences. Notably, the highly asymmetric divergence levels between haplotypes in the four bdelloid rotifers (**Figure 3**) are best explained by a hybrid origin of bdelloids. When tetraploidy was first discovered in bdelloids, it was proposed that it stemmed from either a whole genome duplication or a hybridization event in their ancestor [58]. However, studies of bdelloid rotifers traditionally refer to the divergent haplotypes as “ohnologs” (e.g., [17,18]), which, following the unified vocabulary of Glover et al [57] would imply that the diverged haplotypes are products of a whole genome duplication. However, the most parsimonious explanation for the highly asymmetric divergence of the different bdelloid haplotypes is a hybrid origin. Referring to the diverged haplotypes as homoeologs therefore appears more appropriate. Our analyses also indicate that the allelic heterozygosity varies extensively among bdelloid rotifer genera. Divergence is very low in *Rotaria* (0.49% in *R. magnacalcarata* and 0.125% *R. macrura*) but relatively high in *A. vaga* (2.4%) and in *A. ricciae* (5.5%). There is currently no good explanation for the higher heterozygosity in

A. ricciae compared to A. vaga, furthermore kmer spectra analysis indicates that the genome structure is even more complicated (Supplementary text S4). In *A. vaga* it has been suggested that gene conversion reduces divergence between homologs in some genome regions [17]. It is possible that rates of gene conversion are higher in *Rotaria*, for unknown reasons. Independently of the mechanisms causing the differences between bdelloids, it is important to note that with such low levels of divergence between homologs, there can be no strong genome-wide 'Meselson effect' in bdelloid rotifers (see also [17]). It remains possible that the subset of genomic regions with divergence between homologs in *Adineta* feature allele phylogenies as expected under the 'Meselson effect'. This is the case in the asexual unicellular eukaryote *Trypanosoma brucei gambiense*: some genome regions feature high heterozygosity and allele phylogenies as expected under the 'Meselson effect', while others are largely homozygous [59]. Again, it remains unknown why there is such extensive heterogeneity in divergence across the genome in this species. A possible explanation is that the heterozygous genome regions are the consequence of ancient introgression, and that gene conversion rates are low in such regions because of their very high heterozygosity (see **Conclusions**).

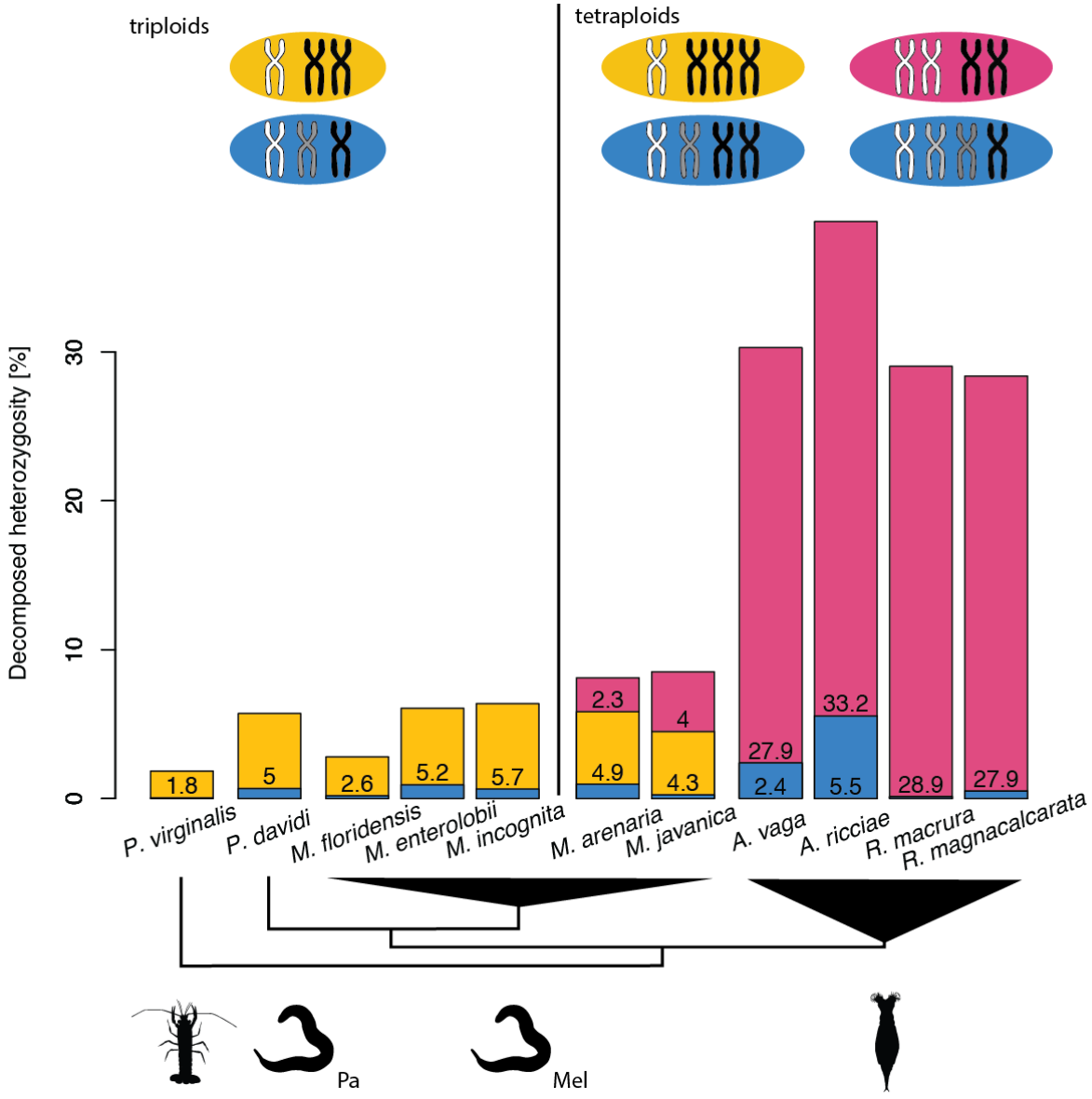


Figure 3: **The relative heterozygosity structure in polyploids.** Biallelic loci are indicated in yellow or pink: yellow when the alternative allele is carried by a single haplotype (AAB or AAAB), and pink when both alleles are represented twice (AABB). Loci with more than two alleles are indicated in blue. The homoeolog divergences in bdelloid species were estimated using genome assemblies, while the remaining values were estimated using kmer spectra analysis (see Methods for details).

Palindromes and gene conversion

Palindromes are duplicated regions on a single chromosome in reverse orientation. Because of their orientation, palindromes can align and form hairpins, which allows for gene conversion within duplicated regions (**Supplementary FIgure 3**). Palindrome-mediated gene conversion was shown to play a major role in limiting the accumulation of deleterious mutations for non-recombining human and chimpanzee Y chromosomes [60–62]. Indeed, approximately one third of coding genes on these Y chromosomes occur in palindromes, and the highly concerted evolution of palindromic regions indicates that the rates of gene conversion are at least two orders of magnitude higher in the palindromes than between homologous chromosomes. The reports of palindromes in the genomes of the bdelloid rotifer *Adineta vaga [17]* and the springtail *Folsomia candida [23]* led to the hypothesis that palindromes could play a similar role in asexual organisms – reducing deleterious mutation accumulation in the absence of recombination. However, the potential benefit of palindrome-mediated gene conversion depends on the portion of genes in palindromic regions [61]. In addition to identifying palindromes, it is therefore important to also quantify the number of genes affected by palindrome-mediated gene conversion.

Methods for palindrome identification depend on genome assemblies (contrary to the other genome features we re-analysed in our study). Palindromes are less likely to be detected in highly fragmented assemblies, and artificial palindromes can be generated by erroneous scaffolding (see also [18]). Our analyses assume that there are no systematic scaffolding

errors in the published assemblies, meaning that our list of palindromes includes false positives that are generated by mis-assemblies in the published reference genomes. Palindrome identification methods rely on genome annotations, which are available for 23 of the 26 asexual species (all except *D. pulex, A. mellifera capensis,* and *A. rufus*). We screened these 23 genomes for the presence of palindromic arrangements (See **Methods** and **Supplementary Text S2** for details). We identified 19 palindromes in *A. vaga*, 16 in *F. candida,* and one to four palindromes in eight additional genomes (Table 1). Not a single palindrome was detected in the remaining 13 species. The frequency of palindromes had no phylogenetic signal; for example, although we found 19 palindromes in *A. vaga*, we found no palindromes in the three other bdelloid rotifers (in agreement with [18]). There is also no indication for major rearrangements being present solely in very old asexuals; among the very old asexuals, the non-*A. vaga* rotifers along with the *Diploscapter* nematodes have either no or only a single palindrome.

Adineta vaga and *F. candida* are the only two species with more than 100 genes potentially affected by palindrome-mediated gene conversion, but even for these two species, the overall fraction of genes in palindromes is very small (1.23% and 0.53% respectively). The fraction of genes in the other seven species ranges between 0.01% and 0.16%, suggesting that palindromes do not play a major role in the genome evolution of any of the asexual lineages analyzed. Our findings substantiate the conclusion of a previous study [18] that major genomic rearrangements and the breaking of gene syntenies do not occur at high rates in asexual organisms. They appear

to occur at rates similar to those known in recombining genome portions of sexual species [63,64].

Table 1: Palindromes in asexual genomes. Only species with at least one palindrome detected are listed in the table. Rows in bold highlight species with more than 100 genes detected in palindromes.

Species	Palindromes detected	Potentially affected genes	Fraction of genes [%]
P. formosa	1	2	0.01
A. vaga	**19***	**636**	**1.29**
O. biroi	2	6	0.04
F. candida	**15***	**152**	**0.53**
M. belari	2	6	0.02
D. pachys	1	2	0.01
M. incognita	1	26	0.06
M. arenaria	3	38	0.04
H. dujardini	1	8	0.04
R. varieornatus	4	22	0.16

* The detected number of palindromes in these species exceeds the number reported in the corresponding genome articles (17 in *A. vaga* and 11 in *F. candida*). This is because we included individual genes in palindromic arrangements, whereas the original genome studies only included genes if they were in palindromic synteny blocks of at least five genes. See also **Supplementary Text S2**.

Mitotic gene conversion can also occur outside of palindromic regions, for example when double-stranded DNA breaks are repaired using the homologous chromosome as a template [65,66]. This can, in theory, contribute to the loss of heterozygosity under all forms of asexuality, but mitotic gene conversion rates have only rarely been studied in asexual species – or sexual ones for that matter. Gene conversion rates are estimated differently in different studies and are therefore difficult to compare: in the water flea *D. pulex*, they were estimated to amount to approximately 10^{-6} locus^{-1} generation^{-1} [24,25,67], in the amazon molly *P. formosa* to 10^{-8} [16]. Up to 11% of the genome of the nematode *D. pachys [29]* is suggested to be homozygous as a consequence of gene conversion, and studies have also argued for an important role of gene conversion for genome evolution in root-knot nematodes [34] and rotifers [17,18], although no quantitative estimates are available for these groups.

Transposable elements

Transposable elements (TEs) are DNA sequences that can autonomously change positions in a genome via various ‘cut-and-paste’ and ‘copy-and-

paste' mechanisms [68,69]. TEs can invade genomes even though they generally provide no adaptive advantage to the individual carrying them [70–72]. To the contrary, new TE insertions in coding or regulatory sequences disrupt gene functions and cause deleterious effects in the host; only very rarely can specific insertions be co-opted to acquire novel, adaptive, functions for the host [72]. In sexual organisms, TEs can spread through panmictic populations because of their ability to rapidly colonize new genomes [10,73]. At the same time, sexual reproduction facilitates the purging of deleterious TE insertions, because recombination, segregation and genetic exchange among individuals improve the efficacy of selection [74,75]. In the absence of sex, TEs could therefore accumulate indefinitely, which led to the prediction that TEs could frequently drive the extinction of asexual lineages. Only asexual lineages without active TEs, or with efficient TE suppression mechanisms, would be able to persist over evolutionary times (Wright and Finnegan 2001; Dolgin and Charlesworth 2006). Consistent with this view, a study in bdelloid rotifers reported extremely low TE loads [76]. This prompted the authors to suggest that bdelloid rotifers could have been able to persist in the absence of sex for over 40 million years thanks to their largely TE-free genomes.

Our analysis of asexual animal genomes does not support the view that bdelloid rotifers have unusually low TE contents. The TE content of bdelloid rotifers (0.7% to 9.1%) is comparable to other asexual animal taxa (median 6.9%, **Figure 4**), all of which are considerably younger than the bdelloids. Across the 26 genomes, there was large variation in total TE content, overall ranging from 0.7% to 17.9%, but with one species, the marbled crayfish,

reaching 34.7%. Nevertheless, the abundance of TEs in asexual animal genomes appears to be generally lower than in sexual species, which range typically from 8.5-37.6% (median: 24.3%) [77]. Whether this difference is indeed driven by asexuality remains an open question as TE loads are known to be highly lineage-specific [20,78]. Furthermore, we annotated TEs in each genome via homology searches in general databases (see methods). This can result in an underestimation of TE loads relative to annotations based on species-specific TE libraries especially in remote lineages as rotifers and tardigrades. However, this is unlikely to be the sole reason behind low TE content of asexuals reported in our study since the methods we used allowed us to identify more TEs than most of the individual genome studies (FIgure 4 and Figure 1). Specifically, most studies estimate TE loads from genome assemblies, which underestimates TE loads because regions with high repetitive contents are generally not assembled.

In addition to other lineage-specific characteristics, the cellular mechanisms underlying asexuality could also affect TE loads. For example, most forms of meiotic asexuality can allow for the purging of heterozygous TE-insertions, given the loss of heterozygosity between generations **(Box 2)**. Barring potential gene conversion events, this form of purging cannot occur under mitotic asexuality. However, in the genomes analyzed here, we did not find any effect of cellular mechanisms on TE loads (**Supplementary FIgure 4**), likely because the expected effect of the cellular mechanisms is very small relative to lineage-specific mechanisms. Moreover, host TE suppression mechanisms can contribute to the inactivation and subsequent degeneration

of TE copies over time, independently of the cellular mechanism of asexuality [72,79].

Two asexual animals clearly stand out (**Figure 4**), one for very low TE content (the rotifer *A. ricciae;* <1% of the genome) and one for very high content (the marbled crayfish *P. virginalis* >34%). There is currently no known mechanism that could help explain why *A. ricciae* differs so extensively from other bdelloid rotifers. In the case of the marbled crayfish, it is unknown whether its extreme repetitive content is a heritage from its sexual ancestor or a consequence of a possible hybrid origin with a subsequent burst of TE activity. In the absence of information on TE loads in the sexual relative *P. fallax,* these possibilities cannot be evaluated. More generally, in most studies quantifying TE contents in asexual species, no comparisons to related sexual species are made. In the cases where this was done, no differences were detected [16,20,21,26,35,80].

Independently of the question of whether asexuality affects genome-level TE loads, our dataset should show whether hybrid species have higher TE loads than non-hybrid species. Indeed, TE activity in hybrids is expected to be high because of mismatches between species-specific TEs and silencing machineries [13,15,81,82]. However, we do not find any difference in TE content according to hybrid vs intraspecific origin of asexuals (**Supplementary FIgure 4**).

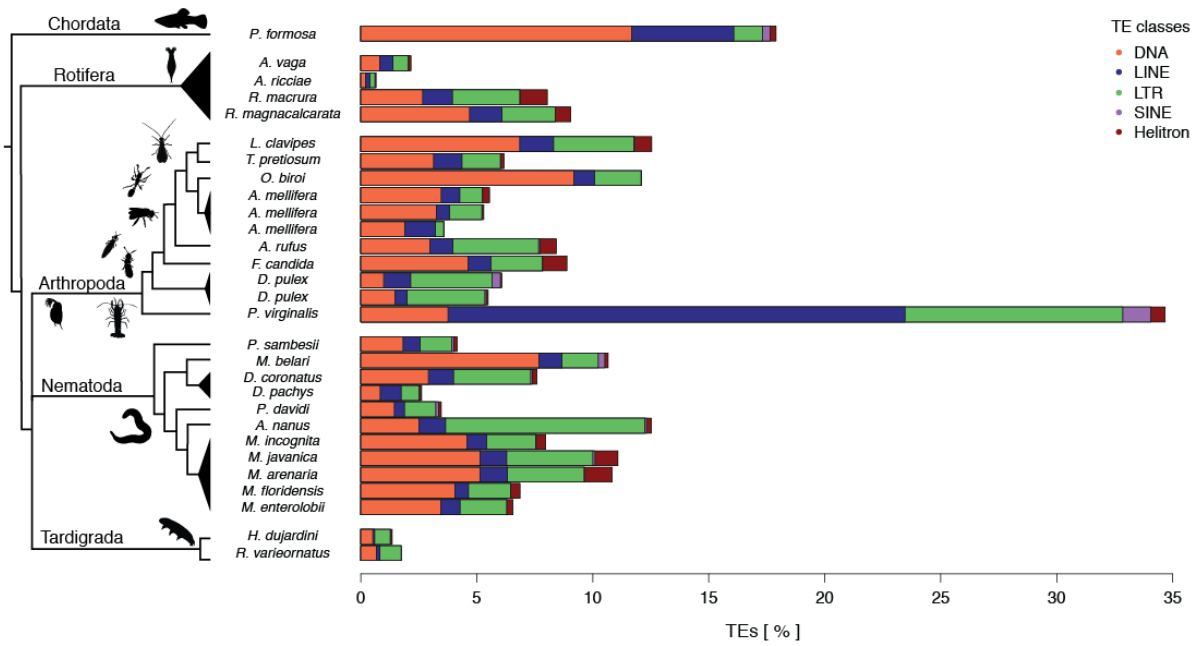


Figure 4: Percentage of transposable elements (TEs) in asexual genomes. Both the TE load and frequency of TE classes vary substantially between individual asexual lineages. The TE classes are: class I “cut-and-paste” DNA transposons (DNA), and class II “copy-and-paste” long interspersed nuclear elements or autonomous non-LTR elements (LINEs), short interspersed nuclear elements or non-autonomous non-LTR elements (SINEs), long terminal repeat elements (LTR), and rolling-circle elements (Helitron).

Horizontal gene transfer

Asexual species could harbour many genes acquired via horizontal gene transfer (HGT) as a consequence of relaxed selection on pairing of homologous chromosomes (see also section **Gene family expansions**). It has also been proposed that HGTs represented an adaptive benefit which allows for the long-term maintenance of asexuality [87]. Indeed, bdelloid rotifers have been reported to carry an unusually large amount (6.2% - 9.1%; Figure 1) of horizontally acquired genes compared to sexual lophotrochozoan

genomes (0.08% - 0.7%) [18,88]. Many of these have been reported to have contributed to adaptive divergence between bdelloid rotifer species [89]. However, there are no other ancient asexuals sequenced and evaluating the role of HGTs in the long-term persistence of asexuality is therefore not possible. In more recent asexuals, reported levels of HGT appear mostly low, e.g. in *Panagrolaimus* (0.63% - 0.66%) and in two tardigrade species (0.8% - 0.97%) [18,30,37]. The only genome with a high reported fraction of HGT (2.8%) outside of the rotifers is the springtail *F. candida [23]*.

We systematically estimated the percentage of non-metazoan HGT candidates (HGT_c) in the 23 of the 26 asexual species with available gene annotations using a sequence comparison based approach, following (Nowell et al. 2018). For each species we compared the set of annotated genes to the UniRef90 database to identify genes of likely non-metazoan origin (Suzek et al. 2015). We considered non-metazoan genes as HGT candidates only if they were on a scaffold that also encoded at least one gene of unambiguous metazoan origin, to control for potential contamination in the genome assemblies (see Methods for details).

The majority of species showed a low proportion of HGT_c genes, with ~1% HGTc in 20 out of 27 (Supplementary Table 4). In agreement with previous findings, we identified elevated levels of HGT_c in the four bdelloid rotifer species *A. ricciae* (10%), *A. vaga* (10.6%), *R. macrura* (8.4%) and *R. magnacalcarata* (7.2%; Supplementary Table 4). Furthermore, we detected unexpectedly high levels of HGT_c in three of the five hexapod species (the

springtail *F. candida* (6.3%), the ant *O. biroi* (14.7%) and the wasp *T. pretiosum* (10.12%)). To evaluate a potential link between elevated HGT_c levels and asexuality in hexapods we quantified HGT_c in published genomes of sexual species from the same order or same superfamily as the asexual species. A similarly high proportion of HGT_C was found in the genomes of a sexual springtail (*Orchesella cincta*; 4.8%), two ant species (*Harpagoxenus saltator* (9.3%) and *Camponotus floridanus* (11.76%) and the jewel wasps (*Nasonia vitripennis* (11.46%) and *Copidosoma floridanum* (10.51%)), suggesting that high HGT_C is a general characteristic of hexapods and not linked to the switch to asexual reproduction. Finally, it is important to note that the level of detected HGT_C is heavily dependent on the parameters used in the pipeline and the database used to distinguish between metazoan and non-metazoan genes (Supplementary text S5). While our analyses show that asexuality is not associated with high levels of HGT_C , the discovery of potentially high levels of HGT in hexapods requires further validation that is outside of the scope of this study.

Gene family expansions

Most genome papers scan for expansions of specific gene families. Such expansions are then discussed in the light of the focal species' biology. The expansion of specific gene families *per se* is thus generally a species-specific trait [91] that is not related to asexuality. For example, expansions of stress response genes in *M. incognita* [33], *P. davidi* [30], and *R. varieornatus* [38]

were suggested to be associated with the evolution of cryptobiosis in these species. To our knowledge, the only example of a gene family expansion that could be directly associated with asexuality is the diversification of the RNA silencing machinery of TEs in bdelloid rotifers [17]. TEs are expected to evolve reduced activity rates in asexual hosts (see section **Transposable elements**), and an improved RNA silencing machinery could be the mechanism underlying such reduced activity rates.

However, mitotic asexuality might allow for extensive variation in gene copy numbers between homologous chromosomes as a consequence of relaxed constraints on chromosome pairing. Gene family expansions (and contractions) could therefore be more extensive and be retained more frequently in asexual than sexual species. To test this hypothesis, an overall comparison of gene family expansions in sexual and asexual sister species is needed (see **Supplementary Text S3**). Four studies have surveyed gene family expansions in asexual species as well as in (sometimes distantly related) sexual counterparts, but these studies found no differences between reproductive modes [16,21,23,31]. However, only two of the four studies are based on asexuals with mitotic asexuality (i.e., where chromosome pairing is not required), and additional studies are therefore needed to address the question of whether asexuality affects gene family expansions.

Gene loss

Asexual animals are predicted to lose genes underlying sexual reproduction traits, including male-specific traits and functions (e.g. male-specific organs,

spermatogenesis), as well as female traits involved in sexual reproduction (e.g., pheromone production, sperm storage organs) [83]. In the absence of pleiotropic effects, gene loss is expected due to mutation accumulation in the absence of purifying selection maintaining sexual traits, as well as to directional selection to reduce costly sexual traits [84]. Some gene loss consistent with these predictions is documented. For example, the sex determination genes xol-1 and tra-2 are missing in the nematode *D. coronatus* [28]. Furthermore, genes believed to be involved in male functions harbour an excess of deleterious mutations in the wasp *Leptopilina clavipes* [19], which could represent the first step towards the loss of these genes. However, a similar excess of deleterious mutations in genes with (presumed) male-specific functions was not detected in the amazon molly *P. formosa* [16].

Species reproducing via mitotic asexuality are further predicted to lose genes specific to meiotic processes [85]. The genes involved in meiosis have been studied in three of eight mitotic parthenogens, as well as in *Rotaria* rotifers and *Diploscapter* nematodes, whose cellular mechanisms of asexuality are unknown. Most meiotic genes have been found in the four bdelloid rotifers [17,18] and in both species of *Diploscapter* nematodes [28,29]. There was also no apparent loss of meiosis genes in the amazon molly *P. formosa* [16]. As much as the idea is appealing, there does not seem to be any support for the predicted loss of meiotic genes in mitotic asexuals. We note that the lack of our understanding of meiosis on the molecular level outside of a few model organisms (particularly yeast and *C. elegans*) makes the interpretation of gene loss (or absence thereof) difficult. This is best illustrated by the fact that

losses of meiosis genes have also been reported in different sexual species, where meiosis is clearly functional [86].

In summary, some gene loss consistent with the loss of different sexual functions has been reported in several asexual species. However, there is no striking pattern relative to sexuals, and a clear interpretation of gene loss in asexual species is problematic because the function of the vast majority of genes is unknown in these non-model organisms.

Conclusions

We re-analyzed 26 published genomes of asexual animals to identify genomic features that are characteristic of asexual animals in general. Many of the original genome studies highlighted one or a few specific features in their focal asexual species, and suggested that it might be linked to asexuality. However, our analyses combined with reviewing published studies show that none of these genome features appear to be a general consequence of asexuality, given that none of them was systematically replicated across even a majority of analyzed species.

The variation among genomes of asexual species is at least in part due to species- or lineage-specific traits. But variation among the features detected in the published single-genome studies is also generated by differences in the methods used. Such differences are often less obvious, yet they can be critical in our assessment of genome diversity among animals. In this work we thus re-analyzed several key genome features with consistent methods. To

minimize the effect of differences in genome quality, we have used in priority robust methods, e.g. based on sequencing reads rather than on assemblies. For example, re-estimating heterozygosity levels directly from reads of each species allowed to show a strong effect of hybrid origin, but not of cellular mechanism of asexuality (**Figure 2**). Another advantage of using the same methods for each species is that it diminishes the "researcher degrees of freedom" [92–94]. For example, the analysis of polyploid genomes requires choosing methods to call heterozygosity and ploidy. By providing a common framework among species, we have shown that homoeolog divergence is very diverse among polyploid asexuals.

We have identified hybrid origin as the major factor affecting heterozygosity levels across all asexual animal species with available genomic data. This is consistent with the conclusions of two studies that focussed on individual asexual lineages: hybridization between diverse strains explains heterozygosity in *Meloidogyne* root knot nematodes and in *Lineus* ribbon worms [34,54]. This rule applies more generally to all the species analysed with known transitions to asexuality, but it is important to highlight that all the non-hybrid species in our dataset are hexapods. Thus in principle the low heterozygosity could be a hexapod specific pattern, for example due to high gene conversion rates in hexapods. The taxonomic range of the sequenced species is wide but we are missing several clades rich in asexual species, such as mites or annelids [95,96]. These clades would be useful foci for future genomic studies of asexual species. Independently of the findings of such future studies, our results suggest that mitotic gene conversion (that acts

independently of palindromes) plays a significant and highly underappreciated role in the evolution of asexual species of intraspecific origin. For example, it has been argued that one of the main benefits of sex could be the masking of recessive deleterious mutations (referred to as “complementation”) which would be exposed under many forms of meiotic asexuality [97,98]. If gene conversion is indeed pervasive, these arguments would extend to functionally mitotic forms of asexuality. Conversely, high rates of gene conversion could also allow for the purging of deleterious mutations while in the heterozygous state, as in highly selfing species (eg. [99,100]). Such purging could help explain why most of the genome scale studies did not find support for the theoretical expectation that asexual reproduction should result in increased rates of deleterious mutation accumulation (see section **Mutation accumulation and positive selection)**. More generally, given the major differences in genome evolution for asexuals of intra-specific vs. hybrid origin, our study calls for future theoretical approaches on the maintenance of sex that explicitly consider the loss *vs*. the maintenance of heterozygosity in asexuals.

In our evaluation of the general consequences of asexuality, we were not able to take two key aspects into account: survivor bias of asexual lineages, and characteristics of sexual ancestors. How often new asexual lineages emerge from sexual ancestors is completely unknown, but it has been speculated that in some taxa asexual lineages might emerge frequently, and then go extinct rapidly because of negative consequences of asexuality. In other words, asexuals that would exhibit the strongest consequences of asexuality, as

predicted by theoretical models, are expected to go extinct the fastest. Such transient asexuals remain undetected in natural populations, because research focuses on asexual species or populations, and not on rare asexual females in sexual populations. Indeed, most of the species included in our study have persisted as asexuals for hundreds of thousands to millions of years. They might thus be mostly representative of the subset of lineages that suffer weaker consequences of asexuality. Finally, the key constraint for identifying consequences of asexuality is that almost none of the published genome studies of asexual animals included comparisons to close sexual relatives. This prevents the detection of specific effects of asexuality, controlling for the variation among sexual species – which is extensive for all of the genome features we analyzed and discussed in our study. Overall, despite the importance of recombination rate variation for understanding the evolution of sexual animal genomes (e.g., [101,102]), the genome-wide absence of recombination does not appear to have the dramatic effects which are expected from classical theoretical models. The reasons for this are probably a combination of lineage-specific patterns, differences according to the origin of asexuality, and survivor bias of asexual lineages.

Methods

We combined different methods into a complete pipeline that collects published assemblies, sequencing reads, and genome annotation data from online databases, and automatically computes the genome features discussed here. The methods for the different steps in the pipeline are detailed below. The pipeline is available at

https://github.com/KamilSJaron/genomic-features-of-asexual-animals. We used this pipeline to gather and analyze the data for 31 sequenced individuals from 26 asexual species. For some species, additional genomes to the ones we used were available, but we did not include them because of low data quality and/or unavailable illumina reads (this was the case for one sample of *M. incognita*, *M. floridensis* and multiple samples of *D. pulex* [24,33,36]). Overall, the genome features computed were: ploidy, genome size, heterozygosity, haplotype divergence structure, transposable elements/ repeat content, conserved gene content (see **Supplementary Text S3**), horizontal gene transfer, and palindrome abundance.

Core genome features (ploidy, haploid genome size, heterozygosity, repetitive fraction of the genome, and characterisation of TE content) were estimated directly from sequencing reads to avoid potential assembly biases in reference genome-based approaches. The raw reads were publicly available for 29 samples and for three more samples shared by authors on request. We cleaned the raw reads by removing adaptors and low quality bases using Skewer (parameters “-z -m pe -n -q 26 -l 21”) [103].

We used smudgeplot v0.1.3 (available at https://github.com/KamilSJaron/smudgeplot/releases/tag/v0.1.3) to estimate ploidy levels. This method extracts from the read set unique kmer pairs that differ by one SNP from each other. These kmer pairs are inferred to derive from heterozygous genome regions. The sum of coverages of the kmer pairs is then compared against their coverage ratio. This comparison separates

different haplotype structures (**Supplementary FIgure 2b**). The most prevalent structure is then indicative of the overall ploidy of the genome. We used this ploidy estimate in all species, except *A. vaga*. The most prevalent structure suggested that this species is diploid. *A. vaga* is well characterized as tetraploid [58], but we were unable to detect tetraploidy because homoeologs are too diverged to be identified as such by the kmer-based smudgeplot method.

Using the inferred ploidy levels, we then estimated genome size and heterozygosity using GenomeScope 2.0 [104] available at https://github.com/tbenavi1/genomescope2.0. GenomeScope estimates genome wide heterozygosity via kmer spectra analysis, by directly analyzing kmers within the raw sequencing reads. A mixture model of evenly spaced negative binomial distributions is fit to the kmer spectrum, where the number of fitted distributions is determined by the input ploidy. Each distribution corresponds to kmers that occur a given time (e.g. once, twice, etc.) in the genome. Fits are then used to estimate heterozygosity, the fraction of repeats in the genome, as well as the 1n sequencing coverage. The latter is subsequently used for estimation of genome size. The definition of heterozygosity for polyploids is not well established, but GenomeScope 2.0 distinguishes different types of heterozygous loci in polyploids (as shown in **Figure 3**). Specifically, GenomeScope 2.0 utilizes a combinatorial mathematical model to account for how particular nucleotide haplotype structures are related to kmer haplotype structures. Assuming mutations are randomly distributed across the genome, three equidistant haplotypes will

generate the highest fraction of triallelic loci. Conversely, if the divergence is carried by the divergence of a single haplotype, very few or no triallelic loci will be detected.

Kmer spectra analysis is affected by the choice of kmer length. Longer kmers require higher sequencing coverage, but lead to more informative kmer spectra. We have chosen the default kmer size 21 nt for all species except the marbled crayfish, where we chose kmer length 17 nt due to low sequencing coverage.

We quantified transposable elements using DnaPipeTE v1.2 [105]. The method uses the haploid genome size (parameter -genome_size) to subsample sequencing reads to a low coverage of 0.5x coverage (parameter -genome_coverage) at least twice. These subsampled reads are corresponding to overrepresented genomic regions, relative to the genomic, non-repetitive background. These repetitive reads, which are including TEs, are then assembled using an assembler (Trintity) that can deal with uneven coverages and is able to split assembled regions with few differences (including different TE families). Following, the assembled sequences are annotated by homology using a database of known TEs. This subsampling process is repeated three times (parameter -sample_number), and the union of results represents the repeat library. The third sampling round is used to map overrepresented reads back to the identified TE library to calculate the overall TE abundance based on the fraction of reads mapping to TEs (for details see [105]). Our reported values of TE loads include only repeats that

were annotated as TEs, i.e., we did not include ‘unknown’ repeats which consist of tandem repeats (satellite repeats), duplications or very divergent/unknown TEs.

The palindrome analysis was based on genome assemblies and their published annotations, from 27 samples of 22 species (annotations were not available for *D. pulex* and *A. rufus*). We performed collinearity analysis using MCScanX (untagged version released 28.3.2013) [106], allowing even a single gene to form a “collinear bloc” (parameter -s) if there were fewer than 100 genes in between (parameter -m). The output was then filtered to contain only blocs on the same scaffold in reverse order. Furthermore we filtered all homologous gene pairs that have appeared on the same strand. All the remaining blocks are palindromes, blocs built of reverse complementary genes on the same scaffold. See **Supplementary Text S2** for more details.

We assessed the impact of HGT on each asexual genome using a sequence comparison based approach, following (Nowell et al. 2018). For each species, the published set of predicted proteins were aligned to the UniRef90 (analysis in the main text) and UniProtKB/Swiss-Prot (analysis in Supplementary text S5) protein databases downloaded 04/07/2019 and 14/08/2019, respectively (Suzek et al. 2015; Consortium and The UniProt Consortium...). The alignment was performed using DIAMOND “blastp” v0.9.21 (Buchfink et al. 2015) (“--sensitive -k 500 -e 1e-10”). For each protein, the HGT index (h_U) was then calculated as $h_U = B_{OUT} - B_{IN}$, where B_{OUT} is the bitscore of the best hit to a protein of non-metazoan origin within UniRef90 and B_{IN} is the bitscore

of the best hit to a metazoan protein (Boschetti et al. 2012). The proportion of secondary hits that agreed with the designation (metazoan vs non-metazoan) was also recorded as the "consensus hit support" (CHS) (Koutsovoulos et al. 2016; Nowell et al. 2018). To account for the confounding effects of database entries from closely related species contributing to h_U, hits from taxa within the same phylum as the focal taxon were excluded from further analysis. Analyses were also run excluding such hits at the class, order and family level for each species, to test the robustness of the results given this partitioning of the target databases (Supplementary text S5). In each case, a focal protein was designated as a putative HGT candidate if $h_U > 30$, $CHS_{OUT} > 90\%$, and the protein was found on a scaffold that also encoded at least one gene of unambiguous metazoan origin (i.e., $h_U < 30$ and $CHS_{IN} > 90\%$).

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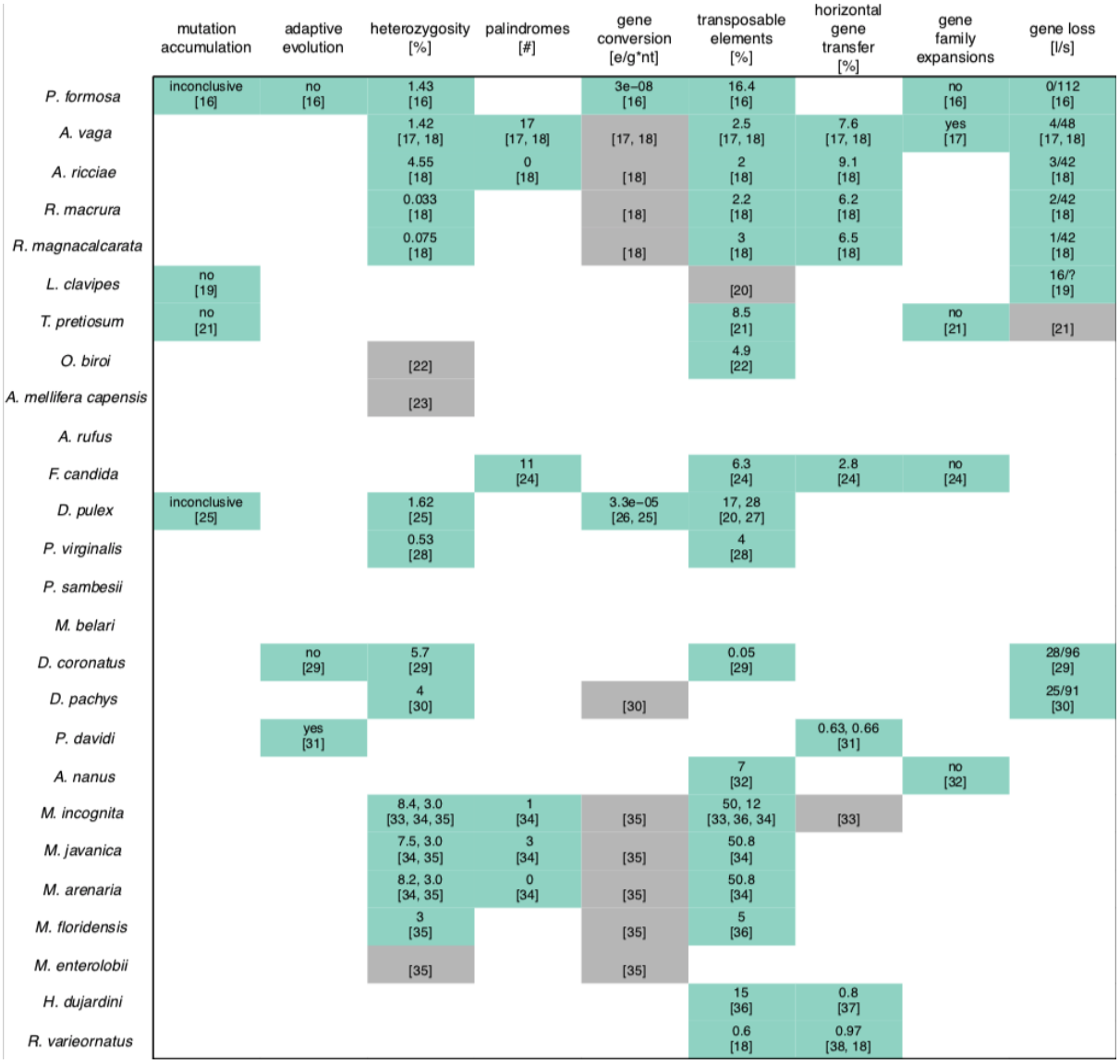
Supplementary materials

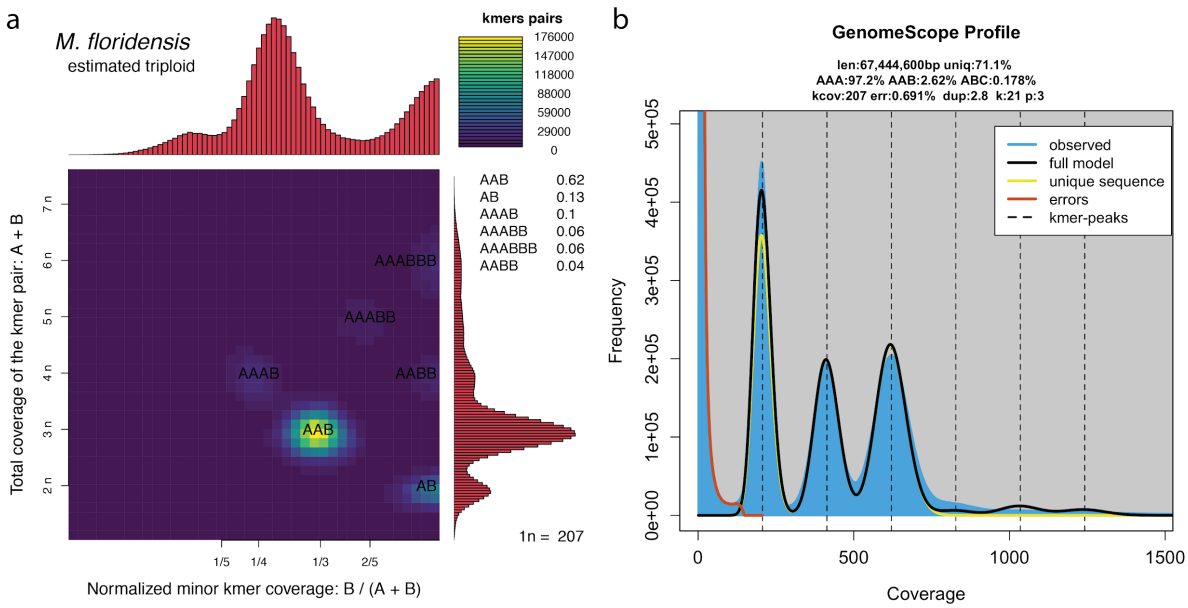


Supplementary Figure 1: Genomic features studied in asexual genomes.

The figure mirrors the data from Figure 1, but adding detailed references to individual studies (numbering corresponding to the references in the text).

S1 Ploidy and reproductive mode of *M. floridensis*

The nematode *M. floridensis* was reported as a diploid species with a mechanism of asexuality functionally equivalent to terminal fusion (absence of the 2nd meiotic division), based on cytological analyses [107]. Our analyses indicate that *M. floridensis* is triploid rather than diploid (**Supplementary FIgure 2**), and the heterozygosity detected in our and previous studies [107] is inconsistent with classical terminal fusion (which should result in largely homozygous genomes, see Box 2 and **Figure 2**). Terminal fusion can be associated with high heterozygosity under inverted meiosis (which is most likely the case in nematodes of the genus *Acrobeloides [108]*. However, inverted meiosis in *M. floridensis* is rather unlikely given that all other meiotic species in the genus have regular meiosis. We therefore believe that the study of Handoo et al is either based on an unusual *M. floridensis* strain that has not been used in any genome study thus far or that the cytology inferred by Handoo et al is not correct. These interpretations are further supported by the fact that Handoo et al report on analyses of large numbers of males of *M. floridensis*, while males are unknown/unusual for the strains used in the genome studies. Unfortunately, it is impossible to evaluate the evidence that supported diploidy and terminal fusion in *M. floridensis* as the study by Handoo et al does not include pictures of karyotypes or egg cells (which is very unusual for this type of research). Given the genomic evidence is very clear, we consider *M. floridensis* to be triploid for all our analyses and the cellular mechanism of asexuality as “unknown”.



Supplementary FIgure 2: Genomic evidence of triploidy in *M. floridensis*.

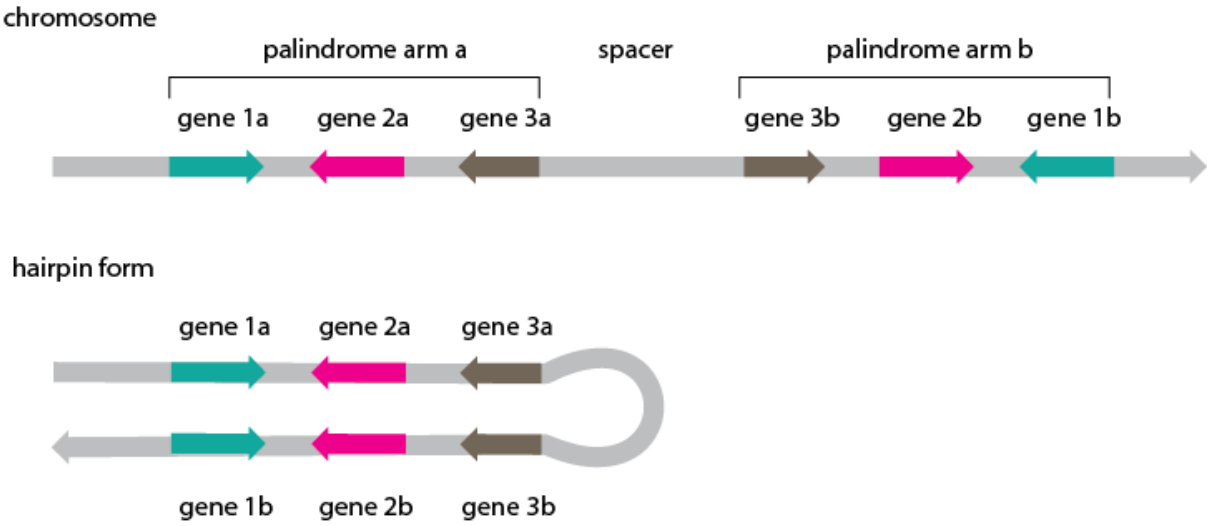
a | the smudgeplot shows dominance of a triploid (AAB) genome structure. The smudges corresponding to higher ploïdies are likely originating from paralogs. The diploid kmer pairs (AB) represent situations where the third allele is diverged from the two more than one nucleotide. **b |** kmer spectra analysis of *M. floridensis* shows a typical triploid genome structure with haploid, diploid and triploid peaks and expected distances from each other.

S2 Palindrome detection

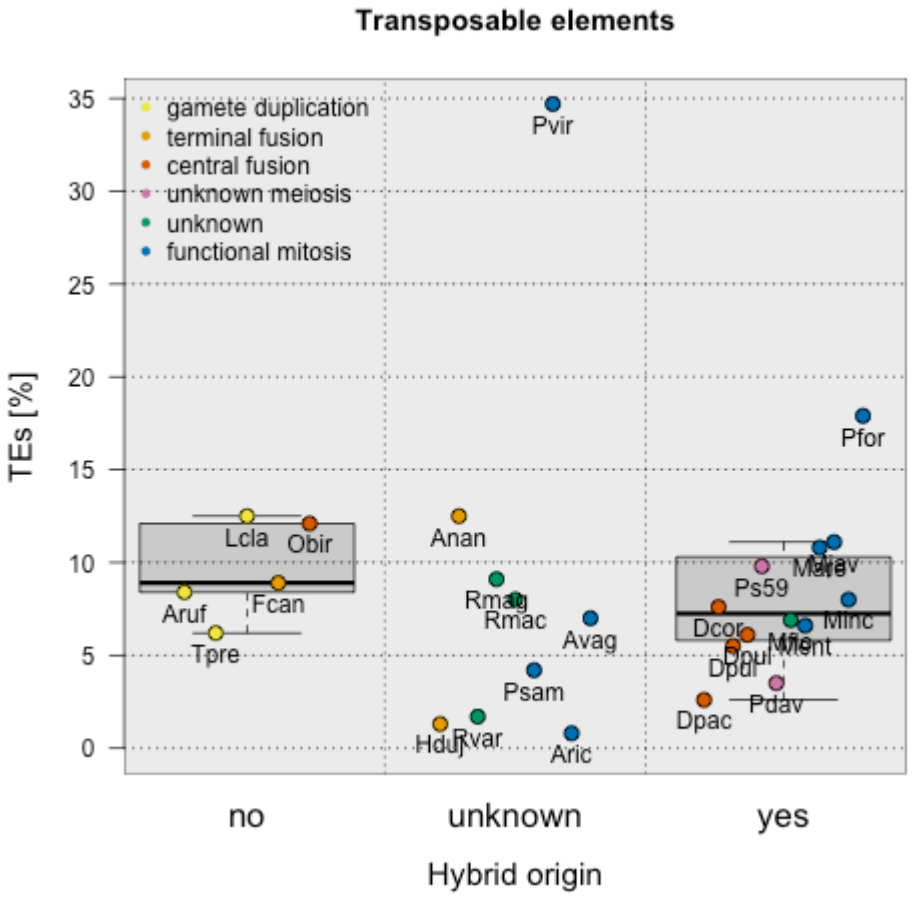
Palindromes are formed of two homologous reverse complementary sequences on the same chromosome (**Supplementary FIgure 3**). Palindromes can facilitate gene conversion and therefore help to escape mutational meltdown via Muller's ratchet [61,62]. To test if they play such a role in asexual organisms we identify palindromes using colinearity analysis imlplemented in the program MCScanX [106]. The default parameters of the software (used in the genome studies of asexual species, personal communication of the authors of [17,23]) define a collinear block as a sequence of at least 5 genes that are no more than 25 genes apart from each other and then search for such blocks with palindromic arrangement. We have reanalysed the genomes allowing for short palindromes of a single gene, because a palindrome could carry fewer than five genes and still be biologically relevant. Detected collinear blocks were filtered to contain only reverse complementary collinear blocks on the same chromosome, since only such structures have the capacity to form a hairpin (**Supplementary FIgure 3**).

We note that it is important to check consistency between the biological interpretation of results, and the methods used to infer them. The bioinformatics pipelines used to detect palindromes are geared towards detecting large repeated blocks with large gaps. We argue that small blocks (as small as one gene), but with no gaps within the inverted repeat may also generate gene conversion. Thus, re-screening the published genomes for

palindromes allowed us to provide a more robust and unbiased view of the importance of palindromes for the evolution of asexual species.



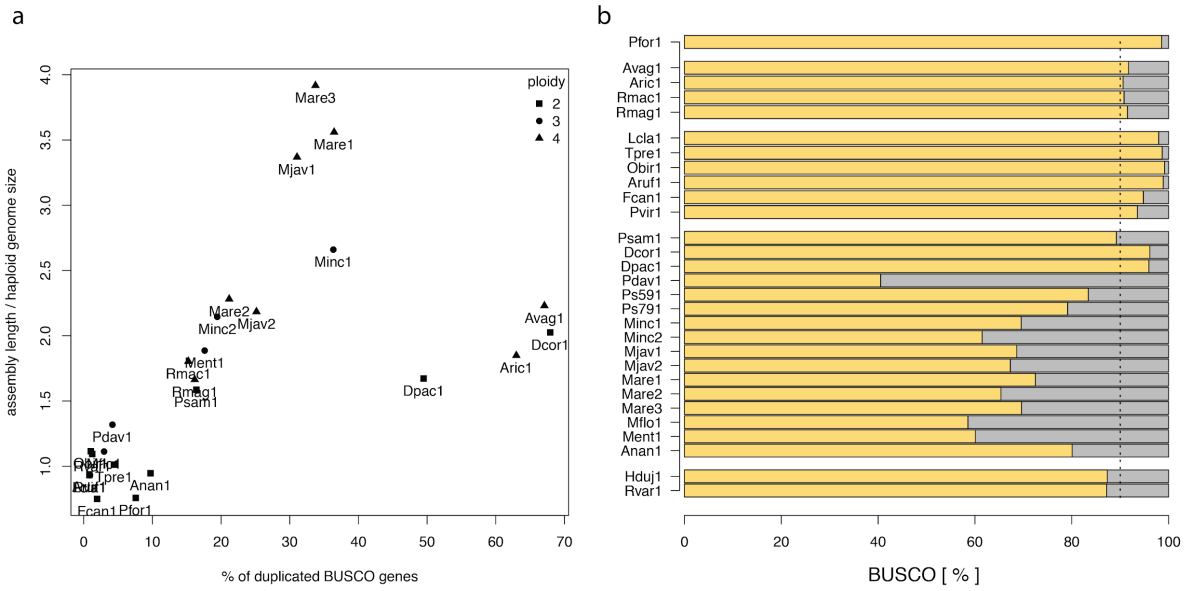
Supplementary FIgure 3: Palindrome structure. The two homologous reverse complementary regions (arms) of a palindrome are located on the same chromosome. This organisation allows for the formation of a hairpin and can facilitate gene conversion between the palindrome arms.



Supplementary FIgure 4: Transposable elements with respect to reproduction mode and hybrid origin. Neither hybrid origin (p-value = 0.36) nor cellular mechanism of asexuality (p-value = 0.84) are strong drivers of the TE content in asexual animals.

S3 Conserved gene content

We aimed to provide insights into gene duplications and losses by quantifying conserved single copy orthologs (BUSCO genes) [109]. BUSCO genes are defined as a set of genes that are present as a single copy in at least 90% of species inventoried in a curated database. All of the species used to build this database are sexual, and we initially hypothesised that both higher duplication rates and gene losses in asexual as compared to sexual species could be reflected in the percentages of missing and duplicated BUSCO genes in the analyzed asexual genomes. However, organisms that are highly heterozygous are prone to separate assembly of homologous haplotypes. In such split genome assemblies, BUSCO genes will falsely appear to be duplicated. To investigate whether split haplotype assemblies are of concern in the analyzed asexual genomes, we deduced the level of haplotype splitting in the assembled genomes by dividing the length of each assembly by the haploid genome size estimated from the read data with genomescope (higher frequencies of separate haplotype assemblies result in higher assembly length to haploid genome size ratios). We indeed found that BUSCO genes appear to be duplicated in genome assemblies consisting of split haplotypes, with the highest level of “artificial duplication” found in polyploid species of hybrid origin (**Supplementary FIgure 5a**).



Supplementary FIgure 5: Conserved single copy orthologs. a | the fraction of duplicated BUSCO genes is correlated to the ratio of assembly length to haploid genome size. **b** I yellow bars show a proportion of BUSCO genes found in individual genomes. The dashed line indicates the expected level.

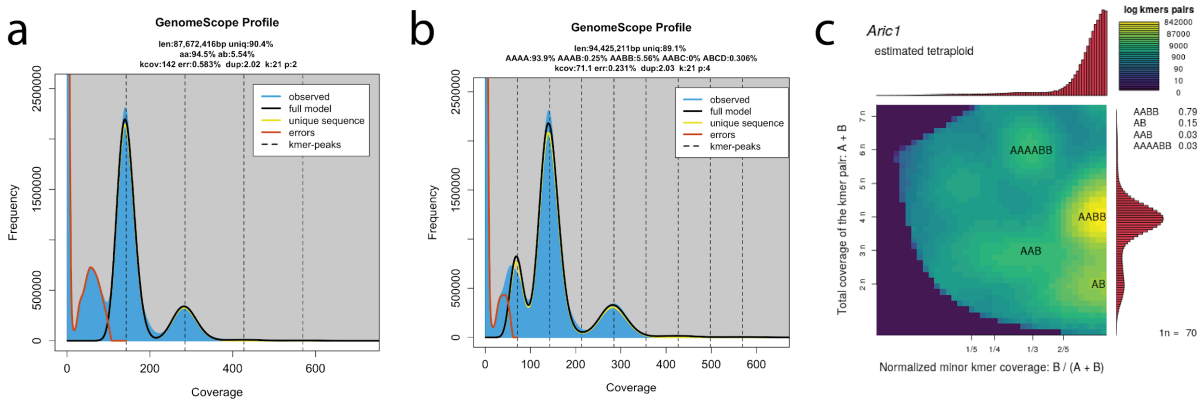
S4 Haplotype structure of *Adineta ricciae*

The maximal genome assembly of *Adineta ricciae* spans 201 Mbp and carries approximately 63,000 genes in an apparent tetraploid structure AABB (Nowell et al. 2018). The divergence between A or B is (from assembly data) estimated 33.21%, while the divergence within A and B is the 5.55% (**Figure 2**). Although A and B have a common ancestor, the divergence reached a level where they share practically no kmers and therefore from a kmer perspective the species is diploid. Finally the genome haploid coverage is expected to be approximately 124x. Using all this information together we might generate an expected kmer spectra. We expect a big haploid peak at 124x, generated from substantial variation within A and B haplotypes. A second peak at ~248x that represents homozygous kmers in A and B. A priori we expect no kmers to be shared between A and B; however, if A and B still share a few regions that could be selectively or mechanistically kept similar we might also find a small peak at ~496x.

The kmer spectra of A. ricciae (**Supplementary Figure 6a**), does show the expected peaks at 142x and 284x that we expected to represent haploid (1n) and diploid (2n) kmers. However, we also observe at least one more peak at 71x, suggesting that our original labeling is shifted by one: 71x representing 1n peak; 142x 2n peak, etc. (**Supplementary Figure 6b**). We verified that the 1n peak contains complementary kmers (**Supplementary Figure 6c**). The kmer spectra is incompatible with the proposed degenerated tetraploid model based by genome assemblies. The 71x peak is even more puzzling if we consider that A. ricciae is considered to have 12 chromosomes. The data

could be compatible only in very unlikely scenarios such as if one chromosome is octoploid and one chromosome tetraploid, or species polymorphism in ploidy levels. This pattern was not replicated in the kmer spectra of *A. vaga* and whatever generates it likely evolved after the split of the two lineages.

Note that the smaller peak in the k-mer spectra is unlikely an artefact of amplification bias during library preparation or mutations in the sequenced clonal population unless the population structure would show exact 50:50 ratio of the two genotypes. New insights could be made with long read sequencing that would allow better separation of the two very closely related sequences (those we detect at 71x peak) or using techniques such as Fugl staining. We are in the process of investigating possible explanations of “the extra haploid” peak in the kmer spectra, however this is beyond the scope of this paper. Here we try to explain why the genomics of *A. ricciae* have no straightforward interpretation.



Supplementary FIgure 6: Genome profiling of *A. ricciae*. a | Genome model used in this study that is compatible with published genome. **b** | The

best fit genome model supporting degenerate octoploid (and practical tetraploid as the high divergence of homoeologs leaves no shared kmers). **c** | smudgeplot of *A. ricciae* supports haploid coverage 70x; supporting genome model shown on panel b. Note that the estimated tetraploidy does not take into account the degenerated state of the genome and therefore implies degenerated octoploidy.

S5 Impact of databases on detection of HGT detection

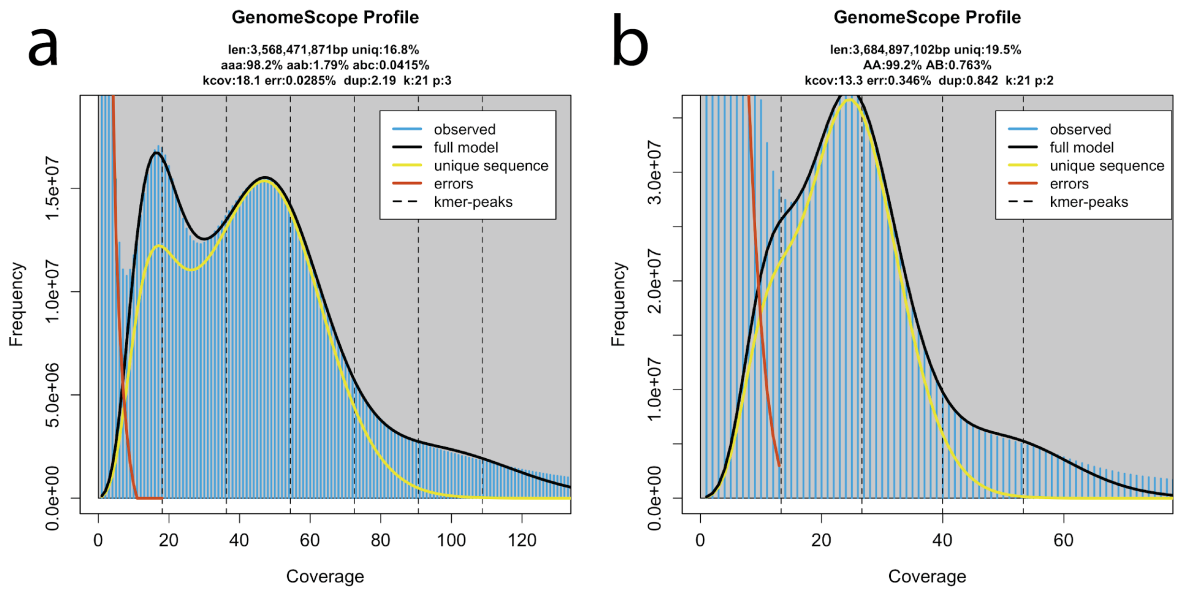
To ascertain the robustness of the detected high levels of HGT_C in the seven species presented in the main text, we also computed h_U based on alignments to the manually curated UniProtKB/Swiss-Prot database. The proportion of HGT_C based on comparisons to UniProtKB/Swiss-Prot was substantially lower in *F. candida* (3.15%), *O. biroi* (0.74%) and *T. pretiosum* (2.36%) than that for UniRef90 (but in some cases higher for certain other taxa, see Supplementary Table S4). Using a conservative database also reduced the estimated levels of HGT_C bdelloid rotifers (3.64 - 5.11%); however, the estimates are still exceeding by far levels of HGT_C detected in any other taxa. The big impact of database can be potentially explained by some taxonomic misclassifications in one or both public databases. On the other hand UniProtKB/Swiss-Prot is more biased towards a handful of model species and do not represent the full species diversity.

Our analysis does not conclusively show absolute levels of HGT in any of the taxa, instead we show how much are such estimates dependent on chosen methodology. Our analysis showed that high levels of HGT are not a general feature linked to asexuality but a clade specific trait of bdelloid rotifers and perhaps of hexapods.

S6 Suggestive hybrid origin of the marbled crayfish

Previously it has been suggested that the triploid asexual crayfish *Procambarus virginalis* is a linage derived solely from diploid sexual *P. fallax* (Martin et al. 2007) and therefore it is autopolyploid rather than allopolyploid. The main arguments for autopolyploidy are that *P. virginalis* and P. fallax are morphologically very similar and that P. virginalis does not carry any trait of any other closely related crayfish. Our analysis revealed two nearly identical genome copies (**Figure 3**) supporting endoduplication as a source of triploidy of the species. However, it also revealed the presence of a highly diverged genome copy, suggestion hybridization between at least highly diverged strains or populations if not species.

Specifically, the estimate of heterozygosity in the asexual triploid *P. virginalis* is ~1.8% (**Supplementary FIgure 7**). Assuming endoduplication, this heterozygosity is generated by the third haplotype, diverged from the two identical copies. If so, we expect ~1.8% to also be the heterozygosity of sexual *P. fallax* individuals, the sexual sister species of *P. virginalis*. The heterozygosity of *P. fallax* is, however, much lower (~0.76%; **Supplementary Figure 7**). This suggests that at least one of the haplotypes was acquired from a more diverged population via hybridization. However, to conclusively determine the origin of *P. virginalis*, we would need to better understand the population genetic diversity of sexual *P. fallax* and haplotype structures in *P. virginalis*.

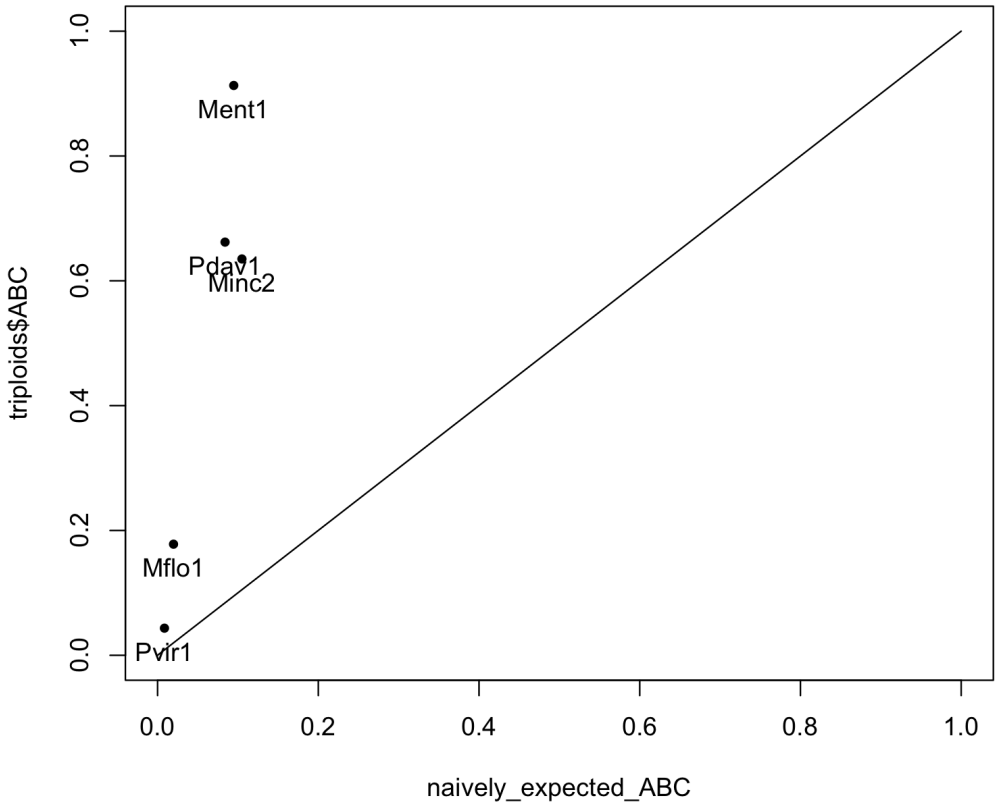


Supplementary Figure 7: Genome profiling crayfishes. a | triploid genome model of asexual *P. virginalis* estimates heterozygosity to 1.79%. **b |** diploid genome model of its sexual sister species *P. fallax* estimates similar genome size, but substantially smaller heterozygosity (0.76%). The quality of fit is less conclusive as the error peak (red) and haploid peak (leftmost black) largely overlap.

S7 Expected fraction of triallelic loci in triploid species

The observed biallelic and triallelic heterozygous loci can be indicative of the genome structure only when compared to an expectation. With an assumption of random distribution of heterozygous alleles we can generate a naive expectation of the fraction of triallelic loci in triploid species. This expectation is dependent on two variables, 1. How equidistant the three genomic copies are 2. The total heterozygosity levels. Consider the two extreme cases. Two identical genome copies result in no triallelic loci, regardless of the total heterozygosity, while three equidistant genomic copies will result in a greater random overlap higher the total heterozygosity is. Consider one reference genomic copy and two other copies with divergence d to the reference. The observed heterozygosity is $2d - d^2$ and the expected triallelic heterozygosity is then d^2.

This expectation does not reflect perfectly the biological reality as genomes contain many regions with elevated or reduced heterozygosity. However, it allows us to compare genomes of various heterozygosity levels. The three species with heterozygosity more equally distributed among the genomic copies (*M. enterobii, M. incognita* and *P. davidi*) exceed the naive expectation by far (Supplementary Figure 8), while *M. floridensis* and *P. fallax* show values much closer to the expectation. The conclusions made in the paper are supported even when we correct for total heterozygosity observed.



Supplementary Figure 8: fraction of triallelic loci compared to naive expectation by a random overlap given the total heterozygosity. We observe two distinct groups of triploid species. The crayfish (Pvir1) and *M. floridensis* (Mflo1) are much closer to naive expectation of triallelic loci, while the three other species show much higher deviation.

Supplementary Table 1: Overview of analysed species. This information was collected directly from the cited literature. References include information regarding cellular mode of reproduction, origin of asexuality and/or the age of asexuality.

https://www.overleaf.com/read/xpzjkrnpnxrm

Supplementary Table 2: Genomic features calculated from raw data. We used unified methods to estimate basic genomic properties directly from sequencing reads. Ploidy was estimated using smudgeplot for all species but *A. vaga* (see section **Heterozygosity structure in polyploids** for details). Genome size, heterozygosity and repeats were estimated using GenomeScope. Repeats denote the fraction of the genome occurring in more than one copy. The classified repeats, TEs and other types of classified repeats, were estimated using DnaPipeTE.

https://github.com/KamilSJaron/genomic-features-of-asexual-animals/blob/master/tables/genome_table_infered_from_reads.tsv

Supplementary Table 3: genome assemblies: size, number of scaffolds, N50, BUSCO, number of annotated genes. Statistics were calculated from the published genome assemblies and genome annotations shared by authors. BUSCO genes were searched using the metazoan database for all the non-nematode species. Nematodes are notoriously known for the high turnover of genes and we therefore used nematode specific BUSCO genes. The number of annotated genes were calculated as the number of lines in the annotation with the tag “gene”.

https://github.com/KamilSJaron/genomic-features-of-asexual-animals/blob/master/tables/assembly_table.tsv

*The number of genes was extracted using the tag “mRNA” since the keyword “gene” was not in the annotation file of *Diploscapter coronatus*.

Chapter 3

Stick insect genomes provide insights into consequences of asexuality

Chapter 3: Stick insect genomes provide insights into the consequences of asexuality

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[*] equal contribution
† authors that had no opportunity to comment on the manuscript.

Abstract

The shift from sexual reproduction to asexuality has occurred repeatedly in animals, but the genomic consequences and the speed at which they emerge remain poorly understood. An excellent model for understanding the consequences of asexuality is provided by the stick insect genus *Timema*, where multiple, independently evolved asexual species with closely related sexual relatives are known. We report ten reference genomes of *Timema* species, five asexual and five sexual ones, and use these in combination with population data to study how asexuality affects genome evolution and polymorphism patterns. We found no systematic effects of the reproductive mode on transposable element load, but we revealed a substantially reduced intra- and intergenomic divergence of asexual compared to sexual species. In asexuals, the rare heterozygous loci were found scattered along chromosomes, and a large portion of them were shared among several individuals, indicating that heterozygosity has been maintained across several generations. This pattern is incompatible with apomixis and therefore homozygosity in asexuals is likely the result of an ameiotic homogenization mechanism such as mitotic gene conversion. Our study is the first to suggest that ameiotic heterozygosity loss might generate genome-wide homozygosity in asexuals.

Introduction

The switch from sexual reproduction to obligate asexuality has occurred repeatedly among animals and is phylogenetically widespread, with several thousand asexual animal species described across the different phyla (Bell, 1982). Asexuality is thought to be favored because it generates a transmission advantage (Smith & Smith, 1978; Williams, 1975), as well as the advantage of assured reproduction when mates are scarce (Gerritsen, 1980; Jain, 1976).

In contrast to the immediate benefits of asexuality, asexuality may be associated with longer-term costs because physical linkage among loci generates selective interference among them (Felsenstein, 1974; Hill & Robertson, 1966; Keightley & Otto, 2006; Muller, 1964). This should translate into decreased rates of adaptation and increased accumulation of mildly deleterious mutations, which may potentially drive the extinction of asexual lineages (Bell, 1982).

Asexuality is also expected to drive major aspects of genome evolution. A classical prediction is that heterozygosity (i.e., intra-individual polymorphism) increases over time in the absence of recombination, as allelic sequences diverge independently of each other, generating the so-called “Meselson Effect” (Birky, 1996; Welch, D. M., Meselson M, 2000). The absence of recombination might also result in an increased frequency of structural variations as found on the human Y chromosome (Jobling et al., 2007).

A key problem in understanding the causes and consequences of the evolution of asexuality has been partitioning the consequences of asexuality from lineage specific effects (Jaron, Bast, Ranallo-Benavidez, Robinson-Rechavi, & Schwander, 2018). Because asexuality is a lineage level trait, partitioning these effects requires replication at the lineage level. However, previous work on genomic consequences of asexuality either did not include independently evolved asexual lineages which prevents distinguishing reproductive mode from lineage level effects, and/or did not include sexual relatives for comparisons which constrains inference of genomic changes that occurred after the evolution of asexuality (Reviewed in (Jaron et al., 2018)).

A unique opportunity to understand the consequences of asexuality for genome evolution is offered by the genus *Timema*, a group of wingless, plant-feeding insects endemic to western North America (Figure 1b). In *Timema* there have been at least seven independent transitions from sexual to asexual reproduction, each representing a biological replicate of asexuality (Figure 1a). Previous research, based on microsatellite markers, has shown that *Timema* asexuals are functionally apomictic, as there is no loss of

heterozygosity between females and their offspring (Schwander & Crespi, 2009). Furthermore, the evolution of asexuality in this group is not associated with hybridization or polyploidization (Schwander & Crespi, 2009). The seven lineages vary in age (Bast, Parker, et al., 2018; Schwander, Henry, & Crespi, 2011), which allows for the consideration of temporal aspects when studying the consequences of the loss of sex.

Here we present a population genomic study of sexual and asexual sister species of Timema stick insects. Although asexual *Timema* is though to be functionally appomictic (Schwander & Crespi, 2009) we found in all asexual species feature genome-wide reduced heterozygosity levels compared to sexual species. In asexual population resequencing data we identified remaining heteorozygous loci shared by multiple individuals. Suggesting that the small portion of the genome remain heterozygous over several generations. We mapped these loci to chromosome-level reference genome of *T. cristinae* and found nearly uniform distribution on chromosomes, suggesting that an ameiotic mechanism causing heterozygosity loss in Timema stick insects.

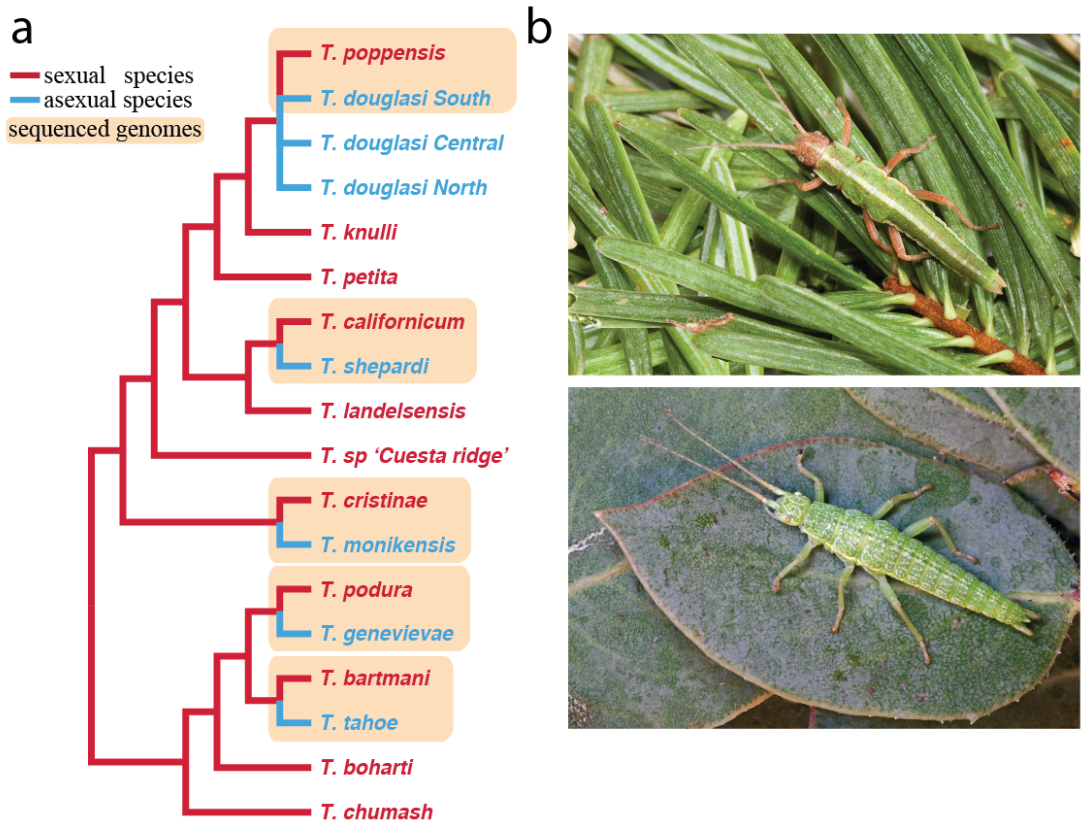

Figure 1: *Timema* genus. a Phylogenetic relationships of *Timema* species (Riesch et al., 2017; Schwander et al., 2011). Species sequenced in this study are highlighted in orange. *Timema* features at least seven transitions from obligately sexual to asexual reproduction (marked by colour). The color code of blue for asexuals and red for sexuals is used in all other figures of this paper. **b** An asexual *T. tahoe* female (top) and *T. shepardi* (bottom) on their host plants. Photos taken by © Bart Zijlstra - www.bartzijlstra.com.

Results and discussion

We sequenced ten species of *Timema* stick insects - five asexual species and their five closest sexual relatives (highlighted in Figure 1a). Assembled genomes were subjected to QC and screened for contamination (see Methods). The completeness of each assembly was assessed by examining how many of the conserved single copy orthologs found across arthropods (BUSCO genes) are present (Waterhouse et al., 2018). We found that between 85.2% to 98.3% BUSCO genes were present in our assemblies indicating that they are largely complete. Only 0.2% to 0.6% of the BUSCO genes were identified in multiple copies, suggesting that the assemblies are mostly haploid. The assemblies span from 1,050 to 1,224 Mbp in size covering 76% - 88.6% of the previously estimated genome size of *T. cristinae* (1,381 Mbp) using flow cytometry (Soria-Carrasco et al., 2014). Although the sequencing coverage was similar across the ten sequenced species (SM Table 2), all five asexual species had both higher continuity (NG50 96 - 226 kbp for asexuals, 4.1 - 112.4 kbp for sexuals) and percentage of identified BUSCO genes (97.1 - 98.3% in asexuals, 85.2 - 97% in sexuals).

Intragenomic variability

The better assembly results for genomes of asexuals than sexuals could be due to systematic differences in repetitive content or heterozygosity (Dominguez Del Angel et al., 2018). To avoid biasing our downstream analyses by the different assembly qualities of sexuals and asexuals, we used a reference-free technique (genome profiling analysis) to estimate basic genomic properties such as genome size, repetitive content and heterozygosity (Vurture et al., 2017). This allowed us to compare the genome characteristics of sexual and asexual *Timema* stick insects directly from sequencing reads. While genome size and overall repetitive content do not show systematic differences between the two reproductive modes (SM Table 3), genome-wide heterozygosity levels of the five asexual species were substantially lower than heterozygosity levels of their sexual sister species (Figure 2a). This pattern is consistent with the idea that heterozygosity variation generates the differences in genome assembly qualities between

sexuals and asexuals (SM Figure 1). All five sexual species feature high heterozygosity levels (0.46-2.31%) but within ranges previously observed in other sexual species (95% of species range from 0.02 - 3.42% , median 0.68%; (Leffler et al., 2012)). We can assume that a high heterozygosity is the ancestral state of the asexual lineages and that it has been reduced during or after the transition to asexuality. Notably, the level of heterozygosity is similar and very low (0.16 - 0.19%) for all asexual species and independent of the heterozygosity levels of the sexual sister species or the age of the asexual lineage. This suggests that the process of heterozygosity loss has already converged to a “heterozygosity loss versus gain by new mutation” equilibrium in all five asexual species.

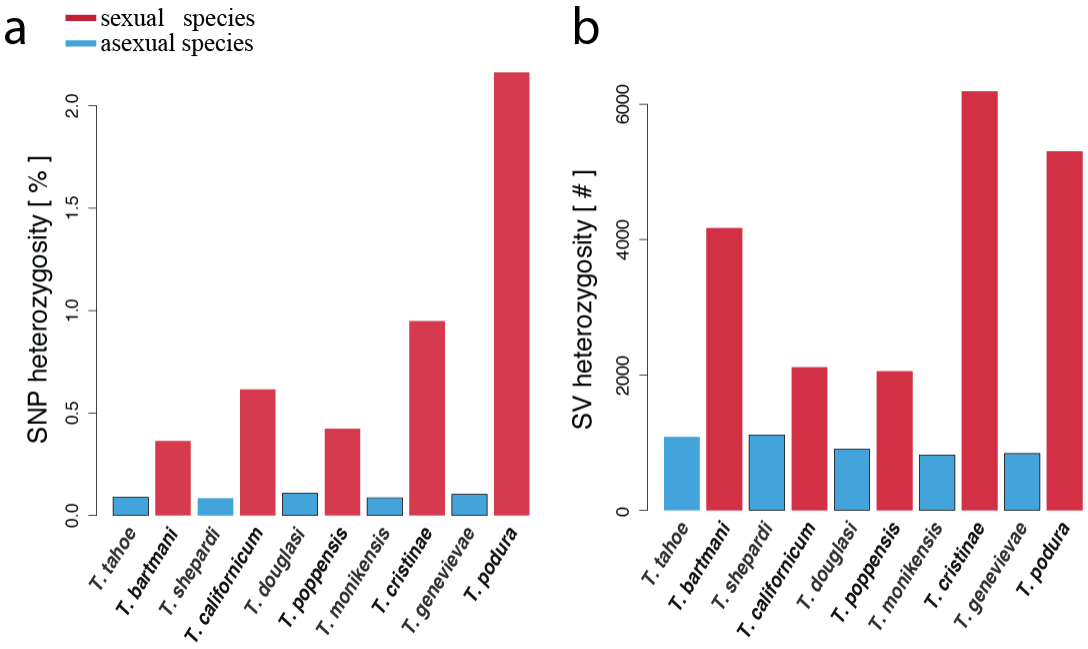

Figure 2: Intragenomic variability in sexual and asexual *Timema* species. Species pairs are sorted by the age of asexuality (youngest to oldest), this order is used in all figures presented. **a** Genome wide SNP heterozygosity levels in the ten sequenced *Timema* species. Regardless of heterozygosity of the sexual sister species, the heterozygosity levels of all the asexual species are very low. **b** Structural variant heterozygosity measured in number of called heterozygous SVs.

It has been previously shown that all five asexual *Timema* species retain heterozygsity between generations at nine microsatellite markers, suggesting

functional apomixis (mitotic asexuality) as their reproduction mode (Schwander & Crespi, 2009). Given this retention of heterozygosity it was unexpected to observe very low heterozygosity levels in all asexual species. The most likely reconciliation of the two observations is that heterozygosity is maintained only in a small portion of the genome, for example centromeric regions. Two non-mutually exclusive hypotheses can explain the heterozygosity loss throughout most of the genome while maintaining it in specific regions. **Extensive rates of gene conversion**, homogenising the genome at a faster rate than mutations occur, can lead to heterozygosity loss over time (Omilian, Cristescu, Dudycha, & Lynch, 2006). The second explanation is that the cellular mechanism of asexuality is in fact **central fusion automixis** under which loci located close to centromeres can retain heterozygosity if recombination rates are low. While intuitively it may seem unlikely that all nine used microsatellite markers are by chance close to centromeres, it is important to consider that centromeric regions are enriched in repetitive sequences and that the markers were selected on the basis that they were polymorphic in asexual species.

To distinguish whether homozygosity in *Timema* asexuals is likely generated by automixis or gene conversion-like mechanisms, we identified heterozygosity for structural variants (SVs). Structural variants are polymorphic nucleotide rearrangements larger than 30 bp, namely insertions, deletions, inversions or translocations. Large SVs might directly affect the heterozygosity distribution in the genome as SVs, and inversions in particular, can obstruct the formation of chiasmata during recombination (Stevison, Hoehn, & Noor, 2011). Central fusion automixis should generate homozygosity for all types of variants, that is, it should have similar consequences for nucleotide heterozygosity (hereby denoted as SNP heterozygosity) as well as for heterozygous SVs. Furthermore, under central fusion automixis, heterozygous loci (both SNPs and SVs) should be located close to centromeres, while homozygous loci cover the rest of chromosomes. On the other hand, we speculate that gene conversion is not necessarily linked to any particular distribution of homozygous and heterozygous loci on the chromosomes and smaller variants might be converted more often than

larger ones as large variants generate problems for homologous pairing. We called heterozygous SVs using paired-end sequencing reads mapped to assemblies (Chen et al., 2016). Consistent with reduced SNP heterozygosity levels in asexual species, we found that the number of heterozygous SVs in asexuals species is substantially lower than in the sexual sister species (Figure 2b). Moreover, levels of nucleotide heterozygosity and heterozygous SVs are remarkably similar between the five independently derived asexual species. A quantitative comparison of SNP heterozygosity and SV heterozygosity is difficult as the number of callable SVs decreases with higher fragmentation of a reference genome which varies a lot among the 10 species (SM table 4). However, our inference that asexuals have substantially reduced levels of SV heterozygosity is robust as improvement of sexual assemblies would lead only to higher humber of called SVs and thus bigger difference between sexual and asexual species.

Heterozygosity in asexual populations

We performed a single nucleotide polymorphism analysis (not presented in this manuscript) and SV analysis of population resequencing data. to fFirstly we verify the detected intragenomic heterozygosity in our reference individuals but also to describe the diversity in asexual populations. We mapped paired-end reads of five resequenced individuals of each species to the corresponding assemblies and called structural variants. We found that the number of detected heterozygous SVs was smaller in the reference individual than in the reseq individuals both in sexual and asexual species (SM Figure 2). This might be due to coverage variation of the haplotypes in the reference individual. The more covered variants of the heterozygous SVs are more likely to be represented in the assembly and therefore the alternative variants have systematically lowered coverage and therefore smaller chances to be recovered. Resequencing individuals are independent of the reference assembly therefore the coverage representation of the reference and alternative variants is random. We confirmed that in each of the six asexual individuals (1 reference, 5 reseq) of all five asexual species we

detected at least 814 SVs, supporting that the reference SV calls were not just an artefact of the analysis.

For the subsequent analyses we merged all detected SVs into population catalogues of SVs. We found that the number of SVs detected in populations differed among species, with sexuals featuring higher population variability (19,700 - 54,300 SVs) than asexual species (3,536 - 13,569 SVs). The structural variations frequency distribution (SVFD) of sexual species reassembles previous report of frequency distributions, whereby the number of SVs decrease with allele frequency (Figure 3a, see {Kidd et al. 2008} for comparison). SVFD of asexual species on the other hand show elevated numbers of SVs for even-numbered allele frequencies (2, 4, 6, etc) in all asexual species (SM Figure 3). This pattern is expected if the population diversity is generated mostly by homozygous alleles. We further decompose variants according to their frequency and the number of individuals carrying them in heterozygous states (Figure 3b). We found that 37.7% - 67.4% of all SVs in asexual populations were detected only in homozygous states and never as heterozygotes, contrasting to sexual species where SVs found only in homozygous states represented only 18.8% - 25.4% of the identified SVs. Furthermore, the proportion of SVs found both in homozygous and heterozygous states was very low for all asexual species (4.4 - 6.1%) compared to sexual species (25.8% - 43%, Figure 3c). Finally among asexual species 31.1% - 61.3% of SVs detected only in a heterozygous state were found in more than one individual suggesting that not all of these variants are recent de novo mutations.

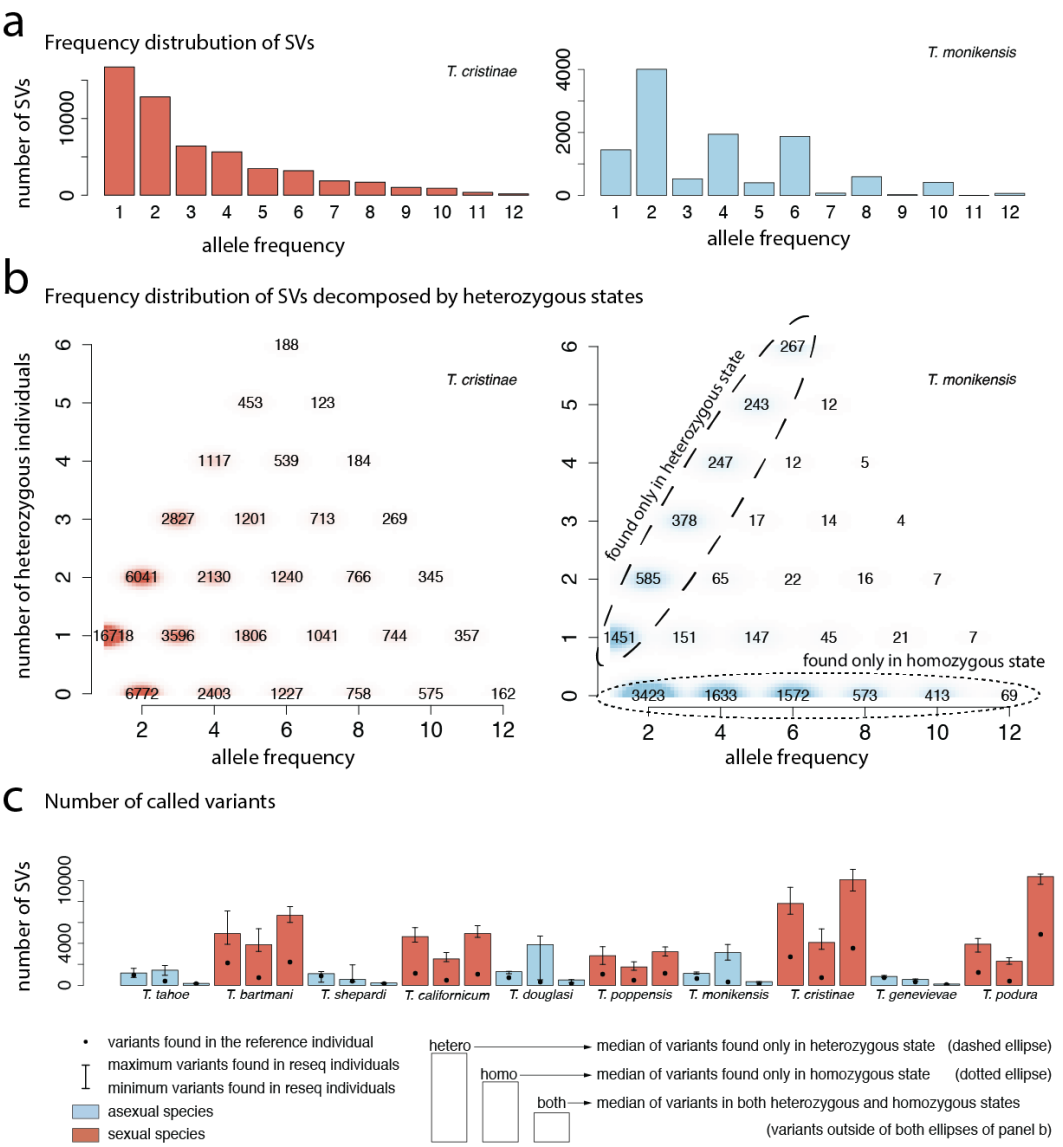

Figure 3: Structural variations in populations. **a** SVFD of one sexual and one asexual species. SVFD of sexual species suggest segregating SVs in contrast to asexual SVFD where even numbers are overrepresented. **b** SVFD decomposed by the number of individuals carrying a heterozygous allele. Sexual species show the expected segregation of alleles. Nearly all SVs identified in asexual species are found either homozygous in all individuals (dotted ellipse) or heterozygous in all individuals (dashed ellipse), where the allele was identified. **c** Overview of all heterozygous, all homozygous and both homozygous and heterozygous loci in all individuals. The three bars represent the three categories: heterozygous - structural variants found only in a heterozygous state (dashed ellipse in c); homozygous - structural variants found only in a homozygous state (dotted ellipse in c); and both - structural variants that are heterozygous in at least one individual while homozygous in one another (all other fields in c). Here we plot the median of number of variants

found in the reseq data. Overall sexual species have more genetic diversity than asexual. Population variability however differs between asexual species.

Localisation of the genomic variability

The loss of heterozygosity in asexuals affects both SNPs and SVs. However, there are some heterozygous SVs in the asexual populations that are avoiding the homogenization mechanism. Both central fusion automixis and extensive gene conversion could produce this pattern. In the case of central fusion automixis, we expect heterozygous SVs to be located close to centromeres, and homozygous SVs to cover the rest of the chromosome. The only heterozygous SVs interspersed with homozygous SVs would be recent de novo mutations. We also expect different SV types (inversions, duplications and indels) to feature similar relative frequencies of homozygous and heterozygous loci. Gene conversion on the other hand is not expected to generate a spatial segregation of homozygous and heterozygous SVs and certain types of structural variants may reduce the probability for local gene conversion events.

We called SVs using individual genome assemblies as a reference. We mapped scaffolds of our assemblies to 12 chromosomes of the previously published reference of *T. cristinae* (Nosil et al., 2018). We aligned between 88.5% and 98.8% of our scaffolds with nucleotide identity ranging from 87% to 97.1% resulting of 31.5 - 73.3% of confidently anchored SVs with an exception of the most fragmented assembly of *T. podura* with only 3.7% of SVs anchored. We mapped only SVs shared by at least two individuals to filter out rare de novo mutations and to reduce possible noise as independently called SVs in different individuals are less likely to be false positives. In all five asexual species, we found heterozygous SVs scattered across the genome without any apparent spatial clustering, interspersed among homozygous SVs (see Figure 4). This pattern is incompatible with any recombination-based mechanism of heterozygosity loss, instead we propose that an ameiotic mechanism of heterozygosity loss, such as mitotic gene conversion, is acting in asexual *Timema* stick insects.

Because *Timema* asexuals are largely homozygous, the loss of heterozygosity between generations cannot (easily) be observed at the genome scale. By contrast, mitotic gene conversion resulting in heterozygosity loss was observed in multiple asexual lineages of *Daphnia pulex* (Flynn, Caldas, Cristescu, & Clark, 2017; Keith et al., 2016; Omilian et al., 2006; Xu, Omilian, & Cristescu, 2011). However *D. pulex* is one of the examples of contageous asexuals, therefore many of the asexual lineages are extremely young with heterozygosity corresponding to the heterozygosity in their recent sexual ancestor. The long term impacts of mitotic gene conversion on heterozygosity in these lineages remains therefore unknown. Constrasting the results for *D. pulex*, no heterozygosity loss was detected in asexual lineages of the water flea species *D. magna* (Dukić, Berner, Haag, & Ebert, 2019). However, the genome-wide heterozygosity of *D. magna* in that study is unknown and may well have been very low. In that case, the heterozygous loci could be portions of the genome not undergoing homogenization, as we suggest is the case in *Timema* stick insects.

In conclusion, our study is the first to support that ameiotic heterozygosity loss might result in overall genome-wide homozygosity with only a small fraction of loci maintaining a heterozygous state. We furthermore propose that retaining SV heterozygosity is either due to mechanistic reasons (too divergent homologs for gene conversion to take place) or selective (homozygous phenotype with dramatic fitness consequences). However further investigations are needed to understand the reasons behind retained heterozygosity of specific SVs in *Timema*.

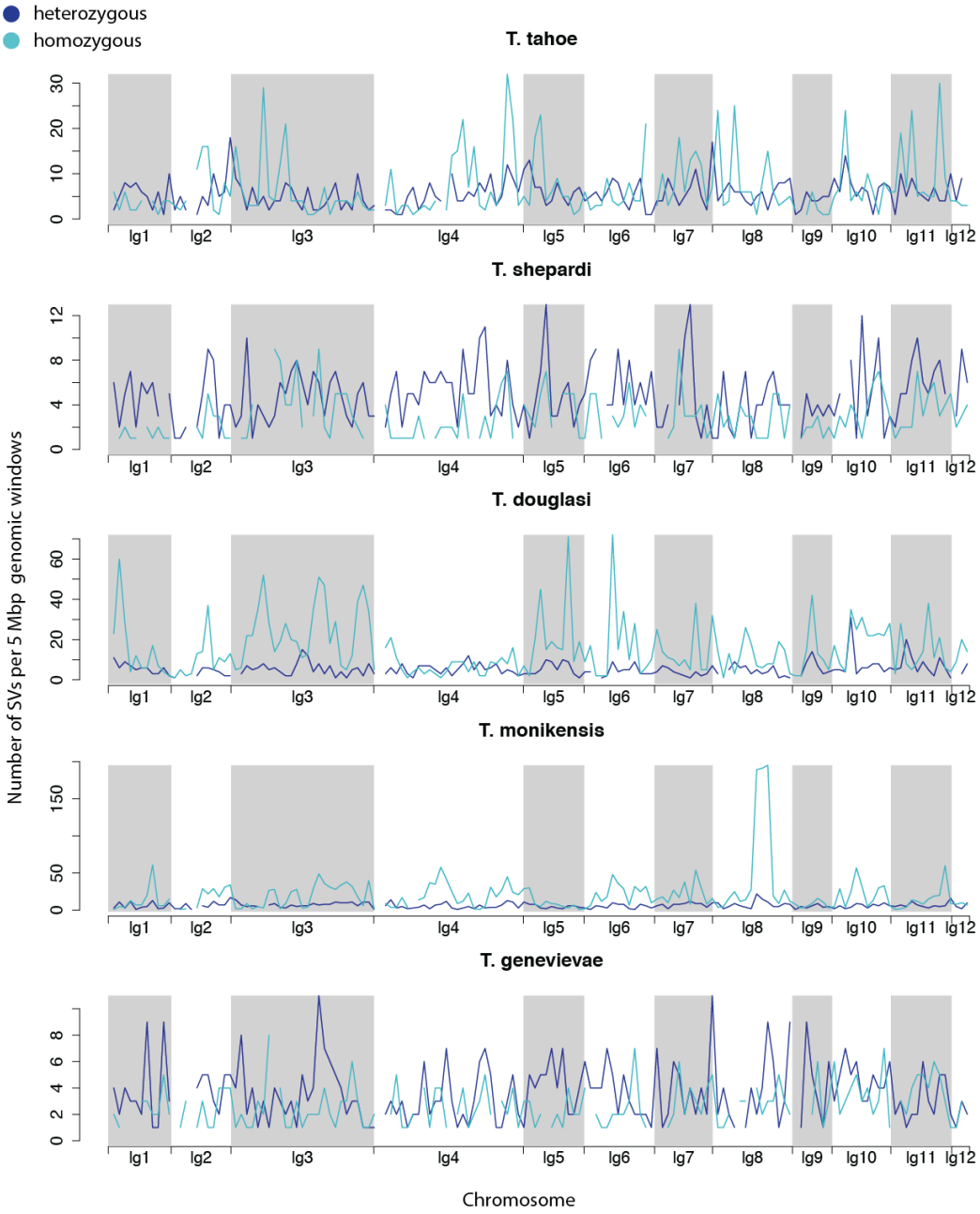

Figure 4: **Genomic localization of non-rare homozygous and heterozygous SVs of asexual species** (sexual species shown in SM Figure 4). Dark blues line represent the number of SVs found only in heterozygous states in a 5 Mbp window. Light blues line represent the number of SVs found only in homozygous states in a 5 Mbp window.

Transposable elements

Upon the loss of sexual reproduction, transposable element (TE) dynamics are expected to change (Bast, Jaron, Schuseil, Roze, & Schwander, 2018; Charlesworth & Langley, 1986; Hickey, 1982). Sex facilitates both the spread and elimination of TEs, whereas in asexuals, TE load might initially increase as a result of weaker purifying selection. However, TE load in asexuals is expected to decrease over time via at least two non-mutually exclusive mechanisms. First, TEs are expected to evolve lower activity over time in asexuals as their interests are aligned with their hosts (Charlesworth & Langley, 1986; Hickey, 1982). Second, TE copies that were purged via excision can re-colonize a sexual but not an asexual genomic background (Bast, Jaron, et al., 2018; Dolgin & Charlesworth, 2006).

We generated species-specific *de novo* TE libraries and quantified total TE loads classified to TE superfamilies in the *Timema* genomes (see **Methods**). We found similar overall repetitive content in the ten species (20 - 23.6%) with significant differences in abundance of TE superfamilies but no significant effect of reproduction mode (TE superfamilies effect $P < 0.001$, *Timema* pairs effect $P = 0.87$, reproductive mode effect $P=0.55$, interaction pairs and mode $P = 0.88$; permutation ANOVA; **Figure 5**).

No difference in TE load between sexual and asexual *Timema* would be expected if TEs would be well controlled already in the ancestor of all *Timema* species without any subsequent TE expansion. However, we detect significant changes of TE superfamilies suggesting at least some recent TE activity in the *Timema* genus. We also did not observe any pattern suggestive of an asexuality age effect in *Timema* (Figure).

Transposable element load of sexual and asexual sister species was measured in water fleas, parasitoid wasps, oribatid mites (Bast et al., 2016; Lindsey et al., 2018), mollies (Warren et al., 2018), root-knot nematodes (Szitenberg et al., 2018) and evening primroses (Ågren, Greiner, Johnson, & Wright, 2015). However, the changes in TE load were found in both directions

and always subtle. The only asexual species featuring substantially lower TE loads in comparison to their closest sexual relatives are bdelloid rotifers. TEs cover 2%–3% genomes of bdelloid rotifers (Nowell et al., 2018), while among monogonont rotifers the TE load ranges 15 - 44% (Blommaert, Riss, Hecox-Lea, Mark Welch, & Stelzer, 2019). Bdelloid rotifers are a very old asexual taxon and the common ancestor of bdelloid and monogonont rotifers dates between 40 - 100 million years ago (Welch, D. M., Meselson M, 2000). As a consequence, it is unclear whether the difference represents an expansion of TEs in monogononts, a contraction of the TE load in bdelloids, or both. In summary, there are at least ten case studies showing a subtle or no difference between sexual and asexual sister species, while there is only a single case of possible reduction of TE load, suggesting that TE dynamics in asexual species are not strongly affected in natural populations, contrasting predictions. The gap between predictions and observations is even more puzzling considering a study of TE activity in sexual and asexual yeast (Bast, Jaron, et al., 2018). After a thousand generations of experimental evolution a significant reduction of TE load in asexual yeast strains was detected, well matching theoretical predictions (Bast, Jaron, et al., 2018; Dolgin & Charlesworth, 2006). How comes then we can not observe a difference in nature after tens or hundreds of thousands of generations?

One possible explanation is that the initial increase of TE load has stronger fitness consequences in natural populations, meaning that asexual lineages derived from sexual ancestors with active TEs go extinct rapidly. As a consequence the only surviving, and therefore observable, asexual lineages are those derived from sexual species with no or very low TE activity. This hypothesis is supported by the overall low activity of TEs in *Timema* genus (Figure 5). However, as we detect the significant changes on superfamily level, explaining TE dynamics in sexual and asexual *Timema* requires further work, such as exploring recent activity patterns of specific TE elements.

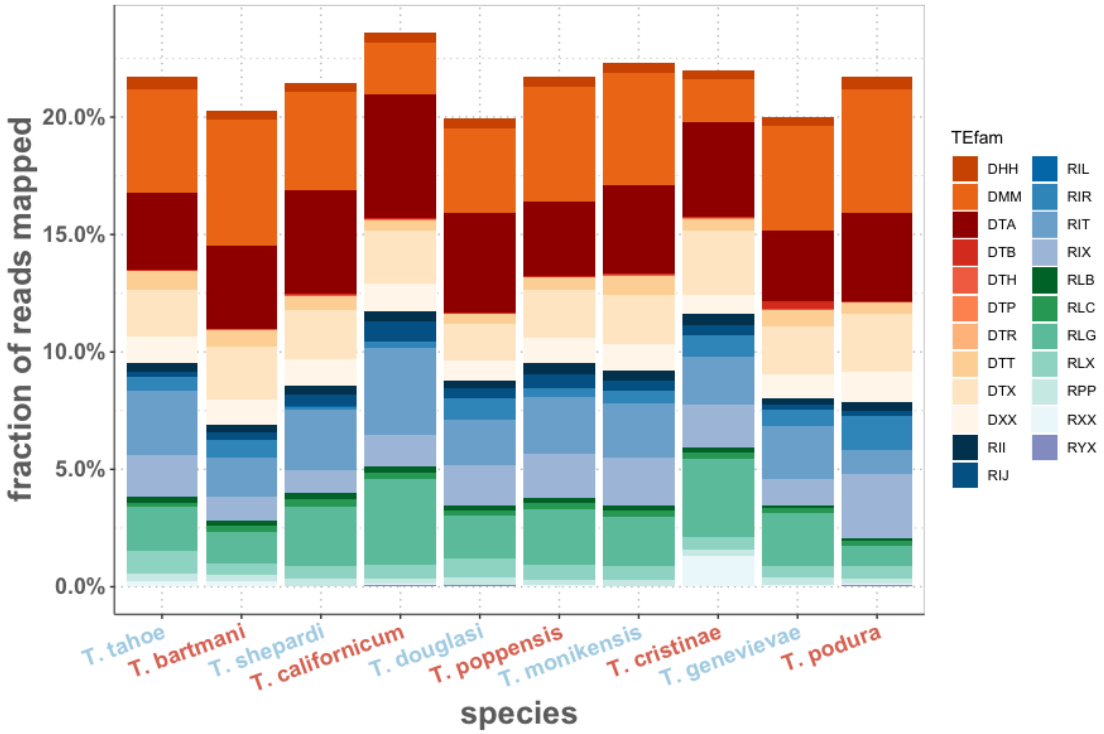

Figure 5: Total TE abundance of the ten *Timema* genomes expressed as the fraction of reads that mapped to a species specific TE library. Species pairs are ranked by age of the asexual lineage from youngest to oldest. TE families are named after Wicker classification (Wicker et al. 2007). The first character corresponds to the TE class (Class I are retrotransposons (R), Class II are DNA transposons (D)), the second character corresponds to the Order (e.g. LTR) and the third to the Superfamily (e.g. *Gypsy*); for example, RLG is a *Gypsy* retroelement. The character X is a placeholder for unknown.

Conclusions

We presented genomes of five independently derived obligately asexual lineages of Timema stick insects, together with their five sexual sister species. All asexual lineages feature substantially lower heterozygosity levels and also a reduction in heterozygous structural variations compared to their sister species. To understand the mechanism behind homogenization of the asexual genomes we analysed structural variations in population genomic data. We found two distinct classes of structural variants in asexual species, variants found only in heterozygous state and variants found only in homozygous states. We found that the two classes of variants are interspersed when mapped to the chromosome-level reference of *T. cristinae*, suggesting

ameiotic mechanisms of heterozygosity loss such as mitotic gene conversion. Our study is the first to suggest that ameiotic heterozygosity loss might result in genome-wide homozygosity.

Methods

All the chosen methods were applied consistently on all ten sequenced species to make results obtained for each genome as comparable as possible.

Sample collection and sequencing

For each of the ten species, the DNA for Illumina shotgun sequencing was derived from virgin adult females collected in 2015 from natural populations in California (SM Table 1). DNA for generating the reference genomes (one female per species) and re-seq data (five females per species) was extracted using the Qiagen Mag Attract de HMW DNA kit, following manufacturer indications. Five libraries were generated for each reference genome (three 2x125bp paired end libraries with average insert sizes of respectively 350, 550 and 700bp, and two mate-pair libraries with 3000 and 5000bp insert sizes), one library (550bp insert size) was generated for each re-sequenced individual.

Assembly method

The total coverage for the reference genomes (all libraries combined) ranged between 37-45x (SM Table 2). Trimmed paired-end reads were assembled into contigs using ABySS (Jackman et al., 2017) and further scaffolded using both paired-end and mate pairs using BESST (Sahlin, Chikhi, & Arvestad, 2016). Scaffolds identified as contaminants were filtered out using Blobtools (Laetsch & Blaxter, 2017). The assembly details can be found in supplementary materials (SM text 1).

Genome profiling

Genome-wide heterozygosity was estimated using genome profiling analysis (Genomescope v1.0.0, (Vurture et al., 2017)). The method is based on a fit of

mixed models to kmer spectra evaluating relative sizes of kmers with coverage corresponding to haploid (heterozygous) and diploid (homozygous) kmers.

Transposable elements

For each species, specific repeat libraries were constructed and annotated (if possible) to the TE superfamily level (Wicker et al., 2007). For collecting repetitive sequences, we used both a raw read based approach (DNAPipeTE v1.2 (Goubert et al., 2015)) and an assembly based approach (RepeatModeler v1.0.8 available at http://www.repeatmasker.org/RepeatModeler/), such that repeats not present in the assembly are represented in the repeat library. The two raw libraries were merged and clustered by 95% identity (the TE family threshold) using usearch v10.0.240 with the centroid option (Edgar, 2010). To annotate TEs larger than 500 bp in the repeat library, we used an approach that combines homology and structural evidence (PASTEClassifier (Hoede et al., 2014)). Because PASTEClassifier did not annotate to TE superfamily levels, we additionally blasted (blastn) the repeat libraries against the well curated T. cristinae TE library generated for (Soria-Carrasco et al., 2014). Blast hits were filtered according to TE classification standards: identity percentage >80, alignment length >80, and the best hit per contig was kept. The two classification outputs were compared and in case of conflict the classification level of PASTEClassifier was preferred. All non-annotated repeats were labelled ‘unknown’. Repeat library header naming was done according to RepeatMasker standard, but keeping the Wicker naming for elements (i.e., Wicker#Repeatmasker, e.g., DTA#DNA/hAT). TE libraries were sorted by header and TE annotations to similar families numbered consecutively.
To estimate the TE load of genomes, we used two approaches: i) repeat masking the assemblies with the species specific repeat library using RepeatMasker v4.0.7 and ii) mapping reads back to the reference genome assemblies and counting the fraction of reads mapping to TEs out of total mappable reads. For the second approach, the gff output of the

RepeatMasker (filtered for TE length of > 80 bp and < 30% divergence) was used together with mapped read alignment as input for htseq-count (Anders, Pyl, & Huber, 2015), to count the number of reads mapping to each genomic location annotated as TE.

Structural variation calling

Methods for SV detection are based either on mapping patterns of paired end reads to a reference or on the analysis of the coverage depth (Medvedev, Stanciu, & Brudno, 2009). One of the problems of SV detection is the lack of a golden standard for the validation of methods, which causes big differences in different benchmarks of SV callers (Alzaid & Badr, 2016; Chen et al., 2016). The lack of information about prediction power of different methods concerns mainly studies dependent on reliable calls of SVs like clinical studies (Sudmant, Alexis, & Burge, 2015). In comparative genomic studies such as ours, the important point is that the SVs detected in different types of genomes can be compared, if there is no systematic bias in the prediction power of the method between species.

We used Manta v1.5.0, a diploid aware pipeline for structural variant calling, which is based on local assemblies of break-end reads (Chen et al., 2016). Manta starts with the construction of a graph of non-adjacent genomic regions using linking information from mapped paired end reads. For the construction of the graph only anomalous read pairs are used, read pairs that have unexpected distribution of insert sizes, wrong mapping orientation or mapping to different scaffolds. Once the graph is constructed anomalous read pairs are translated into sets of SV candidates according to the type of anomaly. The candidates (the translated read pairs) are merged to clusters of candidates supporting the same hypothesis. Each cluster of SV candidate now consists of read pairs supporting the same variant, these reads are then assembled into contig representing the alternative variant. The assembled contig is then aligned to the reference to find the exact borders and size of the inferred SV. We used manta individually to call structural variants in both the reference individuals and reseq data. The six SV calls per species were subsequently merged in unions of population SV calls using SURVIVOR v1.0.2 with default

parameters, merging SV calls of the same type on the same strand with breakpoint no longer than 1000 bp from each other (Jeffares, 2016).

Genome alignment

We mapped our genome assemblies to the reference of *T. cristinae* (BioProject Accession PRJNA417530) (Nosil et al., 2018) using MUMmer (version 4.0.0beta2) with parameter --mum (Kurtz et al., 2004). The alignments were processed by other tools within the package: show-coords with parameters -THrcl to generate tab-delimited alignment file and dnadiff to generate 1-to-1 alignment. We used only uniquely mapped scaffolds to anchor SVs on the *T. cristinae* reference genome.

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Supplementary materials: Stick insect genomes provide insights into the consequences of asexuality

SM Table 1: Origin of biological material. All six individuals per species were always taken from a single location at the indicated coordinates. Red species are reproducing sexually, while blue species are asexual.

Species	Host plant	Coordinates	
		longitude	latitude
T. tahoe	*Abies concolor*	38.7610110	-120.1600530
T. bartmani	*Abies concolor*	34.1700000	-117.0020167
T. shepardi	*Arctostaphylos sp.*	39.1926500	-123.2617833
T.californicum	*Quercus sp.*	37.3431667	-121.6364667
T. douglasi	*Pseudotsuga menziesii*	38.9825500	-123.4697500
T. poppensis	*Sequoia sempervirens*	37.1655167	-122.0155500
T. monikensis	*Cercocarpus betuloides*	34.1148833	-118.8531333
T. cristinae	*Cercocarpus betuloides*	34.5362700	-119.2444300
T. genevievae	*Adenostoma fasciculatum*	38.9957833	-122.9257667
T. podura	*Adenostoma fasciculatum*	33.7976020	-116.7769850

SM Table 2: Sequencing coverages. Read coverage for the reference assemblies of individual *Timema* species was estimated using the haploid genome size of *Timema cristinae* that was previously estimated using flow cytometry to 1.381Gbp (Soria-Carrasco et al., 2014). Red species are reproducing sexually, while blue species are asexual.

species	paired-end			mate-pair		orphans	Total
	Is 350	Is 550	Is 700	Is 3000	Is 5000		
T. tahoe	15	12.2	5.7	4.1	3	3	43.1
T. bartmani	12.3	13.5	3.7	2.7	2.5	2.4	37.0
T. shepardi	12.4	11.6	8.3	3.8	3.6	2.8	42.7
T.californicum	16.4	13.2	8.1	4.4	2.8	3.1	48.2
T. douglasi	13.2	11	8.8	4.3	2.8	2.9	43.1
T. poppensis	12.5	12.1	7.1	2.9	2.8	2.7	40.2
T. monikensis	13.8	12.6	9.6	3.4	4.2	3	46.6
T. cristinae	13.7	10.9	10	4	3.6	3	45.3
T. genevievae	15	13.4	4.3	2.5	5.4	2.8	43.5
T. podura	15.7	10.8	3.1	3.1	2.6	2.3	37.7

SM Table 3: Genome profiling. Stats derived directly from reads using genomescope. Red species are reproducing sexually, while blue species are asexual.

species	Haploid genome size [Gpb]	Repetitive content [%]	Heterozygosity [%]
T. tahoe	1.13	32.7	0.088
T. bartmani	1.15	34.4	0.362
T. shepardi	1.23	35.8	0.082
T.californicum	1.3	40	0.615
T. douglasi	1.26	38.1	0.108
T. poppensis	1.31	41.1	0.423
T. monikensis	1.12	31.4	0.086
T. cristinae	1.11	32.7	0.948
T. genevievae	1.07	29.9	0.103
T. podura	1.04	32.9	2.162

SM Table 4: Genome assembly statistics. Genome assembly statistics of sequenced *Timema* species. Sister species are kept together and species pairs are sorted by age of asexuality. Total sum represents sum of all scaffolds. NG50 was calculated using a flow cytometry genome size estimate of *Timema cristinae* (1.381Gbp). The proportion of conserved single copy orthologs among insects (BUSCO score) was calculated using only scaffolds longer than 1000 bases. N is the proportion of unknown nucleotides (gaps) in the assembly. Red species are reproducing sexually, while blue species are asexual.

species	□ [Gpb]	NG50 [kbp]	BUSCO [%]	Ns [%]	Repeats [%]	TEs [%]
T. tahoe	1.093	125.4	97.4	2.4	39.2	21.7
T. bartmani	1.109	104.8	97	2.6	38.2	20.3
T. shepardi	1.153	103.5	97.6	1.7	39.6	21.4
T.californicum	1.22	66.5	93.6	1.7	40.5	23.6
T. douglasi	1.124	96.3	97.1	1.6	37.7	20
T. poppensis	1.138	39.5	91.9	1.6	38.4	21.7
T. monikensis	1.099	226.3	98.3	1.7	41.2	22.3
T. cristinae	1.178	112.7	96.8	2.3	39.6	22
T. genevievae	1.05	141.4	98	1.6	40.1	20
T. podura	1.105	4.1	85.2	0.4	41.1	21.7

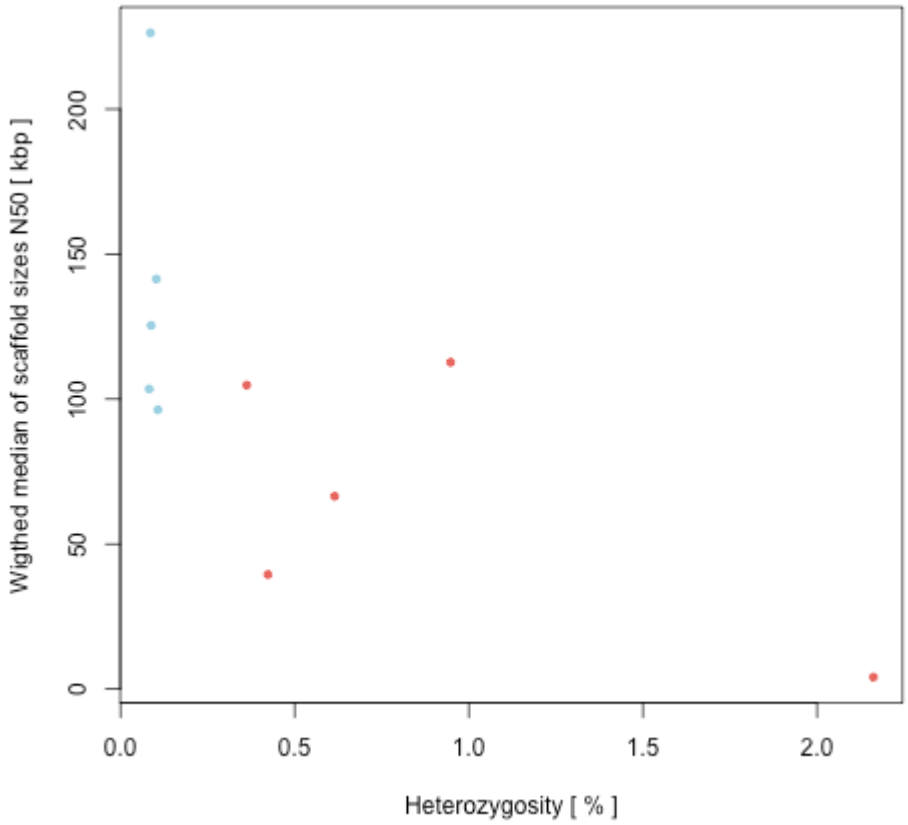
SM text 1: Assembly pipeline.

Here we describe the final pipeline we have used to generate our reference genome assemblies, however we justified each decision by extensive tests of multiple algorithms and/or parameters. Paired-end raw reads were trimmed according to sequencing quality and matches to known Illumina sequencing adapters using Trimmomatic (Bolger, Lohse, & Usadel, 2014). Leading and trailing bases below quality 9 were removed. Reads were scanned using a 4-base sliding window, trimmed when the average quality dropped below 15, and discarded if read length dropped below 96bp (Parameters: PE ILLUMINACLIP:all-adapters.fa:3:25:6 LEADING:9 TRAILING:9 SLIDINGWINDOW:4:15 MINLEN:96). The raw mate-pair reads were de-linked and reverse complemented using NxTrim (O'Connell et al., 2015) with the parameter "--preserve-mp". Unlinked pairs without identified adapter sequence, called unknown pairs, were also considered as valid mate pairs as they had a similar distribution of insert sizes as mate pairs with identified linker sequence.

Filtered paired-end reads were *de novo* assembled using ABySS v1.9.0 (Jackman et al., 2017; Simpson, Wong, Jackman, Schein, & Jones, 2009) with default parameters and k-mer sizes predicted to be optimal using kmergenie (Chikhi & Medvedev, 2014). The k-mer sizes were 83, 87, 83, 87, 83, 89, 81, 81, 65 and 87 for *Timema poppensis*, *T. douglasi*, *T. californicum*, *T. shepardi*, *T. cristinae*, *T. monikensis*, *T. barmani, T. tahoe, T. podura and T. genevieve* respectively. Assembled contigs longer than 250 bases were scaffolded using BESST v2.2.5 (Sahlin et al., 2016) with default parameters and gap-filled with GapCloser v1.12-r6, a module of the SOAP package (Luo et al., 2012).

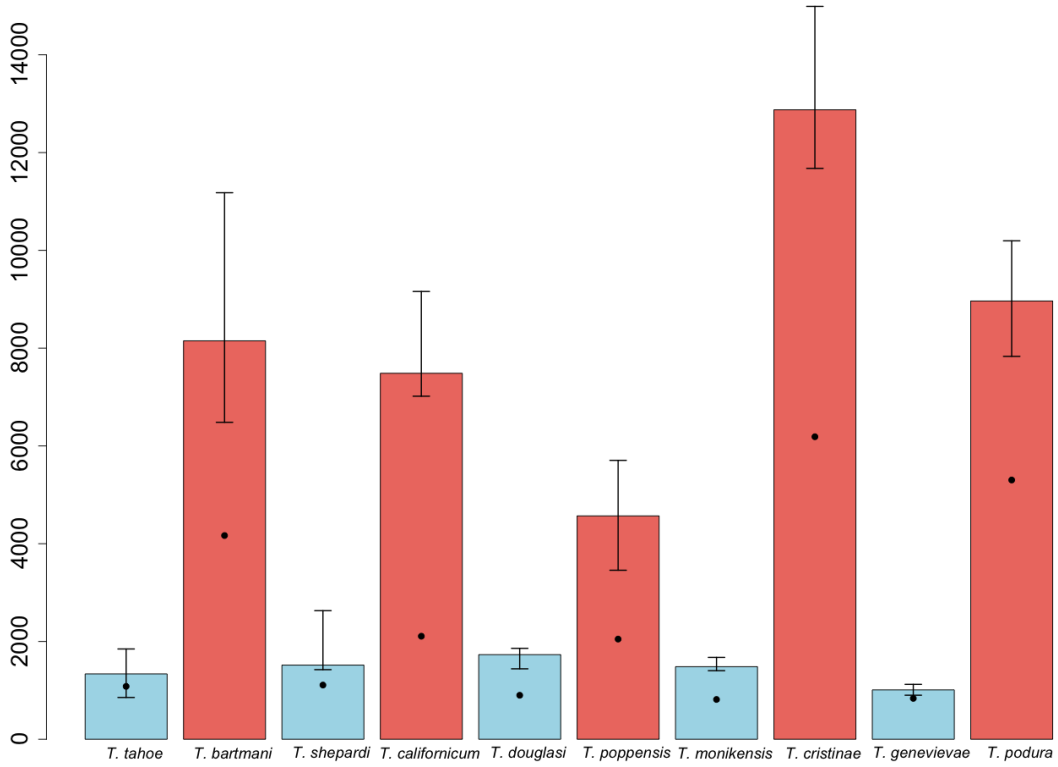
Genome assemblies were decontaminated using BlobTools v0.9.19.5 (Laetsch & Blaxter, 2017). Hit files were generated after a blastn v2.6.0 against the NBCI nt database, searching for hits with sequence identity above 85% and an e-value below 1e-25 (Parameters: -task megablast -culling_limit 5 -evalue 1e-25 -perc_identity 85). Scaffolds without hits to metazoans were removed from the assemblies. The genome assembly completeness was assessed with BUSCO v3.0.2 (Waterhouse *et al.* 2017) against the insecta_odb9 lineage.

SM figure 1: Correlation of heterozygosity and assembly continuity (N50)



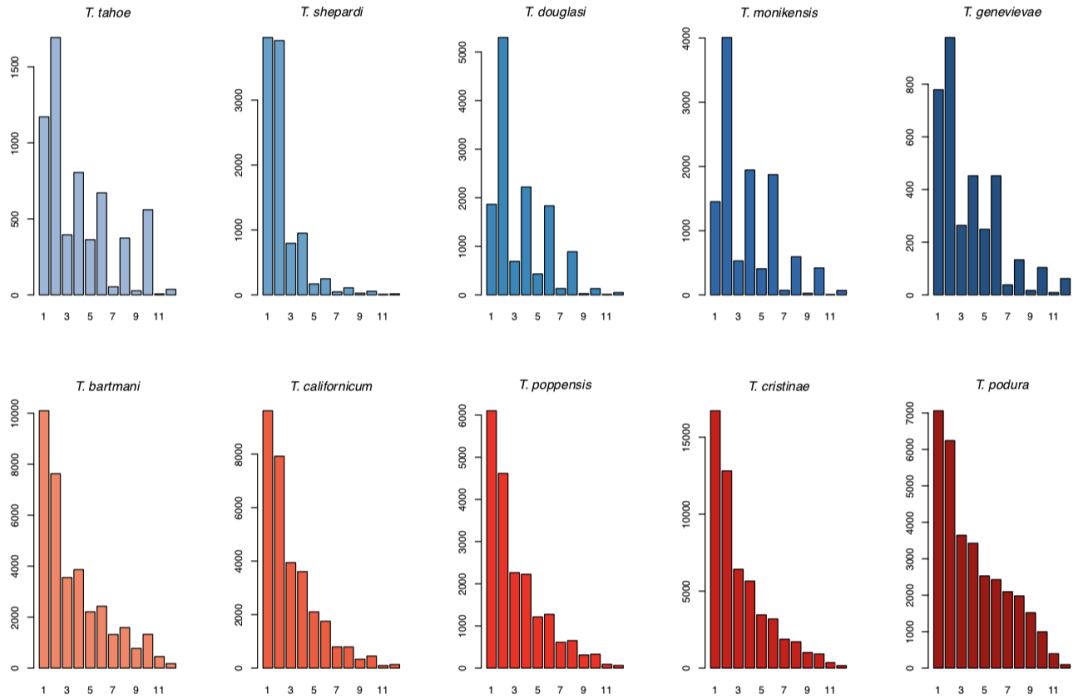
SM figure 1: Higher heterozygosity levels are associated with lower continuity. Although this is a strong association, heterozygosity explains only 36.53% of the variability in N50, suggesting there are also other unidentified factors involved.

SM figure 2: intragenomic SV heterozygosity in population data



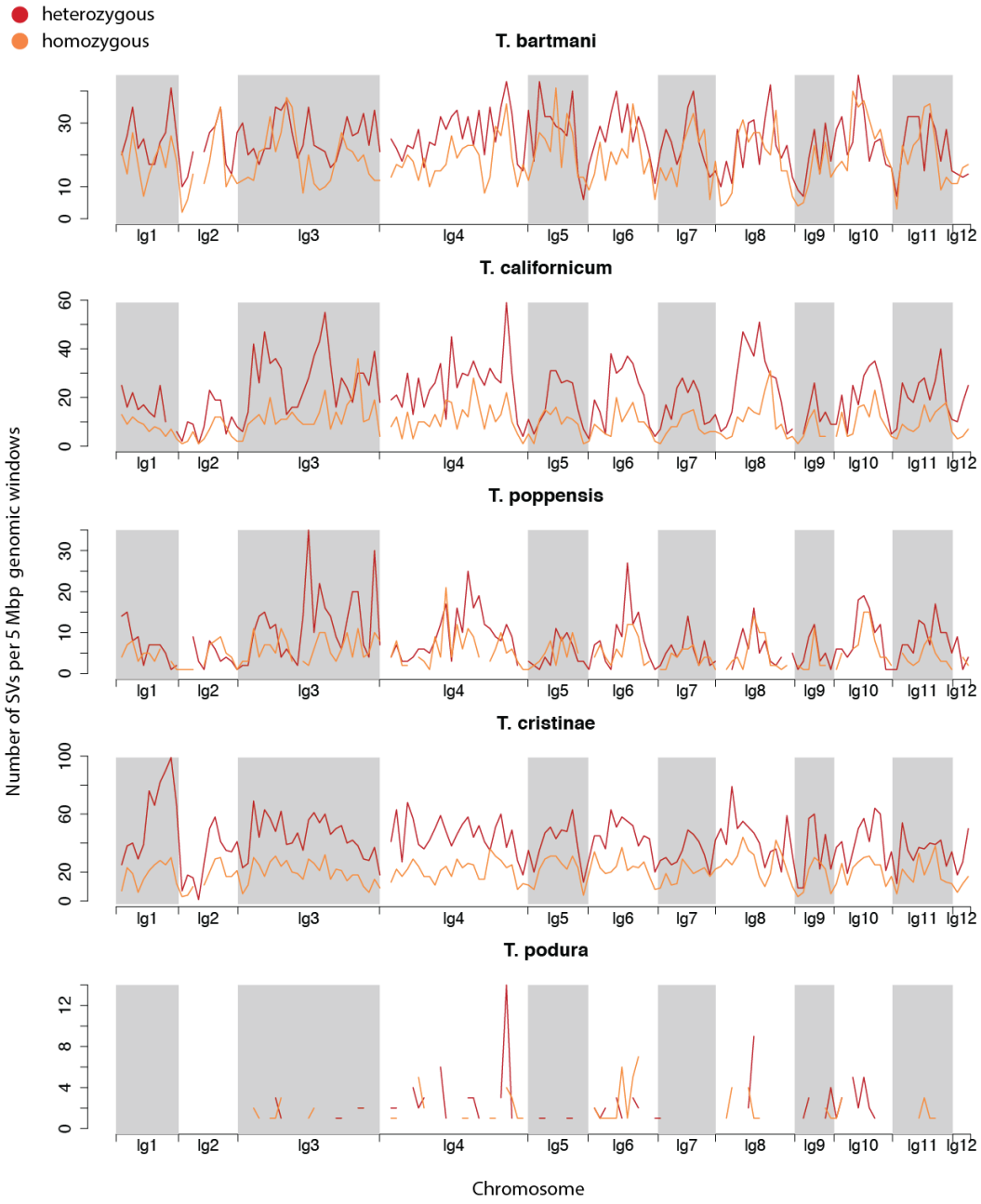
SM Figure 2: Number of heterozygous SVs in population resequencing data. The bars and whiskers represent median, minimal and maximal number of heterozygous SVs calls in reseq data. Black dots are numbers of SVs called in the reference individual.

SM figure 3: Structural variant frequency density for all ten species



SM Figure 3: Site frequency spectra for all ten species. All five asexual species (in blue) show an enrichment of homozygous loci in the SFS, but some (*T. monikensis* and *T. douglasi*) of the asexual species more than others (*T. shepardi*).

SM figure 4: Overview of SVs decomposed per type



SM Figure 5: localization of SVs in genomes of sexual *Timema* stick insects. Although SVs show non-uniform distribution, the localization of homozygous and heterozygous SVs are interspersed similarly to asexual species (Figure 4). The poor representation of *T. podura* stems from the poor mapping of SVs in the heavily fragmented assembly of *T. podura* (SM Table 4).

Chapter 4

Asexual reproduction drives the reduction of transposable element load

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Chapter 4: Asexual reproduction reduces transposable element load in experimental yeast populations

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Abstract

Theory predicts that sexual reproduction can either facilitate or restrain transposable element (TE) accumulation by providing TEs with a means of spreading to all individuals in a population, versus facilitating TE load reduction via purifying selection. By quantifying genomic TE loads over time in experimental sexual and asexual *Saccharomyces cerevisiae* populations, we provide direct evidence that TE loads decrease rapidly under asexual reproduction.. We show, using simulations, that this reduction may occus via evolution of TE activity, most likely via increased excision rates. Thus, sex is a major driver of genomic TE loads and at the root of the success of TEs.

Main Text

Self-replicating transposable elements (TEs) can occupy large fractions of genomes in organisms throughout the tree of life (reviewed in Hua-Van et al., 2011). Their overwhelming success is driven by their ability to proliferate independently of the host cell cycle via different self-copying mechanisms involving ‘cut-and-paste’ or ‘copy-and-paste’ systems. These mechanisms allow TEs to invade genomes in a similar way to parasites, despite generally not providing any advantage to the individual carrying them (Doolittle and Sapienza, 1980; Orgel and Crick, 1980). To the contrary, TEs generate deleterious effects in their hosts by promoting ectopic recombination and because most new TE insertions in coding or regulatory sequences disrupt gene functions (Finnegan, 1992; Montgomery et al., 1991).

Theory predicts that sexual reproduction can either facilitate or restrain the genomic accumulation of TEs, and it is currently unclear whether the expected net effect of sex on TE loads is positive or negative. Sexual reproduction can facilitate the accumulation of TEs because it allows TEs to colonize new genomes and spread throughout populations (Hickey, 1982; Zeyl et al., 1996). Because the colonization of new genomes is more likely for active TEs, sexual reproduction should favor the evolution of highly active TEs (Charlesworth and Langley, 1986; Hickey, 1982), even though increased activity generates higher TE loads in the host genome. At the same time, sexual reproduction facilitates the evolution of host defences and increases the efficacy of purifying selection against deleterious TE copies by reducing selective interference among loci (Agren and Wright, 2011; Arkhipova and Meselson, 2005; Crespi and Schwander, 2012; Wright and Finnegan, 2001). In the absence of sex, reduced purifying selection can thus result in the accumulation of TEs, unless TE copies get eliminated via excision at sufficiently high rates (Burt and Trivers, 2006; Dolgin and Charlesworth, 2006).

Genomic TE loads have been empirically estimated for natural populations of asexual and related sexual organisms, but no consistent difference emerges (Agren et al., 2015; Bast et al., 2016; Jiang et al., 2017; Szitenberg et al., 2016), probably because many confounding factors not related to reproductive mode such as hybridization and polyploidization can affect TE loads and mask the effect of sex (Arkhipova and Rodriguez, 2013; Hua-Van et al., 2011).

Here, to quantify whether the net effect of sexual reproduction on genomic TE loads is positive or negative, we study the evolution of genomic TE loads in experimental yeast (*Saccharomyces cerevisiae*) populations generated in a previous study (McDonald et al., 2016). McDonald et al. maintained four sexual and four asexual strains originating from the same haploid ancestral strain (W303) under constant conditions over 1000 generations. For sexual strains, a mating event (meiosis) was induced every 90 generations. Sequencing of each strain was conducted at generation 0 and every 90 generations prior to mating (for details see **Methods**, and McDonald et al., 2016). In the present study, we use the published Illumina data to quantify TE loads in each strain for each sequenced generation.

TEs in *S. cerevisiae* are well characterized (Carr et al., 2012; Castanera et al., 2016; Voytas and Boeke, 1992). *S. cerevisiae* TEs consist solely of 'copy-and-paste' elements that are flanked by long terminal repeats (LTRs) and are grouped into the families *Ty1-Ty5* (Voytas and Boeke, 1992). The 12.2 Mb genome of the studied yeast strain comprises approximately 50 full-length, active *Ty* element copies, and 430 inactive ones (Carr et al., 2012). Inactive copies include truncated elements as well as remnants from TE excisions (i.e., solo-LTR formation;Carr et al., 2012). Excisions occur by intra-chromosomal recombination between the two flanking LTRs of a TE, and result in the removal of protein-coding genes that allow for transposition.

Using different computational approaches to quantify genomic TE loads in experimental yeast strains, we show that sex is required for the success of TEs, as TE loads decrease over time under asexual reproduction. For the first approach, we quantified total TE loads without distinguishing between active

and inactive TEs. This was done by computing the fraction of reads that mapped to a curated *S. cerevisiae* TE library (see **Methods**) for each yeast strain and sequenced generation. This analysis revealed that the total TE load in sexual strains remained constant over 1000 generations, but decreased in asexual strains over time (resulting in a total reduction of 23.5% after 1000 generations; generation effect $P < 0.001$, reproductive mode effect $P = 0.081$, and interaction between generation and mode $P < 0.001$; permutation ANOVA, **Figure 1-figure supplement 1**). For the second approach, we focused on , full-length TE copy insertions, because only those are active and can lead to increased genomic TE loads over time. Detecting specific TE insertions by aligning short-read data to a reference genome is difficult and associated with a detection bias towards TEs present in the reference genome. Moreover, because sequencing was done with population pools and not individual clones within populations, it is not possible to analyse turnover or activity of TEs within specific genomic backgrounds. Instead, we analyzed the presence versus absence of specific TE insertions in each population over time. With a pipeline that combines different complementary approaches (Nelson et al., 2017, see **Methods**), the available sequencing data allowed us to detect 24 out of the 50 full-length insertions that are present in the reference genome of the ancestral strain at the start of the experiment (generation 0). As with the first approach, we found that the number of (detectable) full-length TE copies remained constant in sexual yeast strains, but decreased in asexual strains over time (generation effect $P = 0.006$, reproductive mode effect $P = 0.033$, and interaction between generation and mode $P < 0.001$; permutation ANOVA). In asexual strains, the estimated

average number of full-length TEs decreased from approximately 50 to 41 over 1000 generations (**Figure 1**).

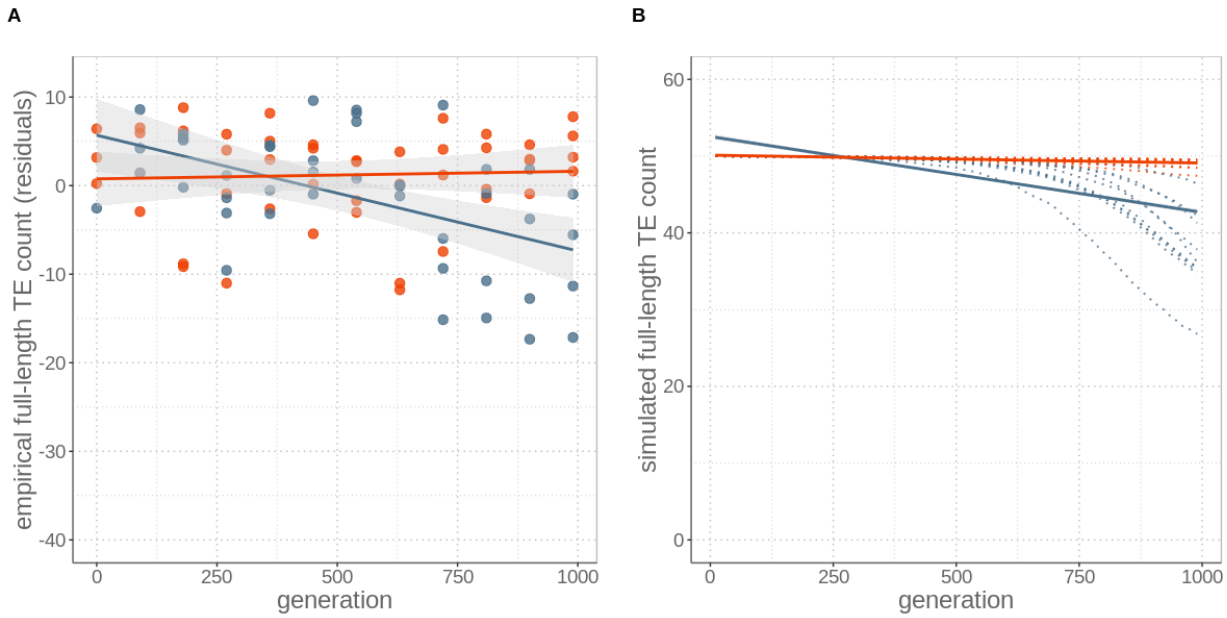

Figure 1. Sex maintains constant TE loads through time, while its absence leads to TE copy number reduction, for both (**A**) empirical data and (**B**) simulations including an allele modifying TE activity rates. **(A)** Number of full-length TE copies inserted in genomes of four replicates of otherwise identical occasionally sexual (red) and wholly asexual (blue) yeast strains over 1000 generations of experimental evolution. Numbers are expressed as residuals, since the TE detection probability depends on sequencing coverage (Figure 1-figure supplement 2). **(B)** Individual-based simulations for studying the TE load dynamics expected under sexual and asexual reproduction with ten replicates (red and blue dotted lines). The simulations are parameterized with yeast-specific values and include a modifier allele . For both (A) empirical and (B) simulation data, asexuals lost about nine active, full-length TEs by generation 1000. Lines represent linear regression for sexuals (red) and asexuals (blue) and the grey areas represent 95% CI.

This decrease could be generated by either increased TE excision rates in asexual as compared to sexual yeast, reduced transposition rates, or a combination of both mechanisms. To evaluate the relative importance of the two mechanisms, we estimated the number losses of TEs present in the ancestral yeast strain, as well as the number of novel insertions, at each asseyed generation (**Figure 2**). These analyses revealed that 'ancestral' TE insertions are lost at a higher rate in asexual than sexual strains (generation effect P = 0.002, reproductive mode effect P = 0.027, and interaction between generation and mode P < 0.001; permutation ANOVA), while we detected similar numbers of novel TE insertions (indicating similar transposition rates) under both reproductive modes (generation effect P = 0.338, reproductive mode effect P = 0.271, and interaction between generation and mode P = 0.599; permutation ANOVA). Taken together, our empirical observations indicate that even very rare events of sex (here just 10 out of 990 reproduction events) are sufficient to maintain genomic TE loads, while asexuality results in the reduction of TE loads..

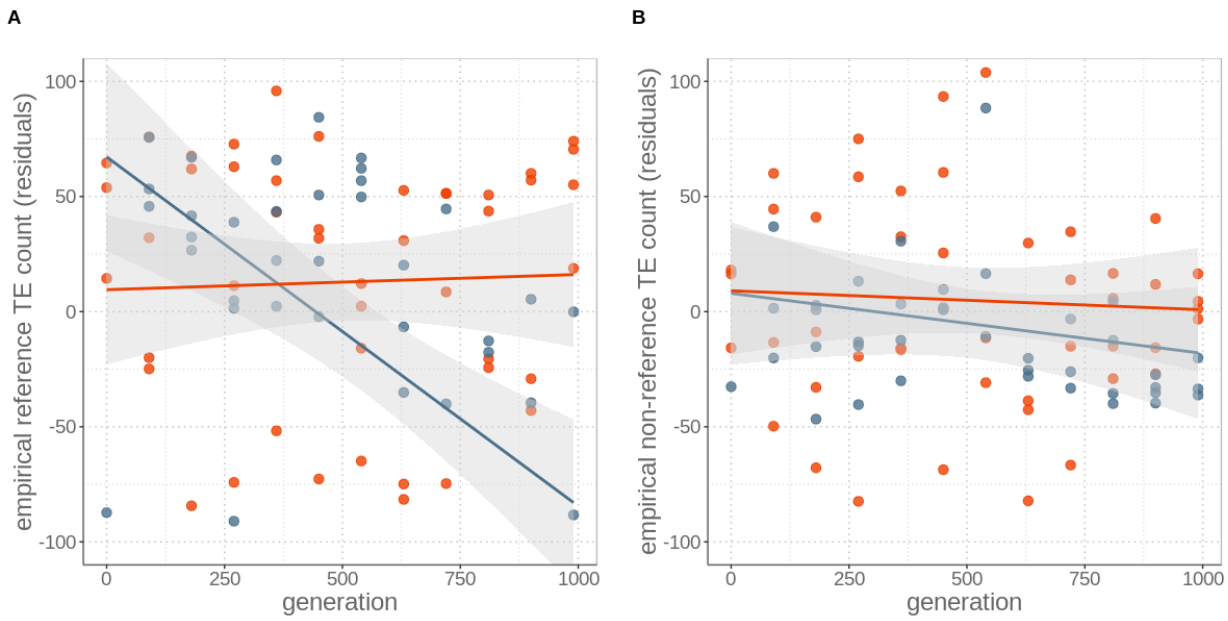

Figure 2. Decrease of insertions in asexuals over time is largely due to loss of 'ancestral' reference insertions (**A**) rather than novel insertions (**B**). Count of all TE insertions, irrespective whether full-length TE, solo LTR, truncated elements or other types in genomes of four replicates of sexual (red) and asexual (blue) yeast strains over 1000 generations of experimental evolution. Numbers are expressed as residuals, since TE detection probability depends on sequencing coverage. Lines represent linear regression for sexuals (red) and asexuals (blue) and the grey areas represent 95% CI.

The parallel reduction of TE loads in different asexual strains suggests that the evolution of reduced TE activity (the ratio of transposition to excision) in asexual strains influences genomic TE loads more strongly than purifying selection, which should act to reduce TE loads most effectively in sexual strains. To evaluate whether these findings are plausible, we tested whether the net loss of TEs under asexualitly is predicted by a simple model of TE dynamics. As explained above, different theoretical approaches have shown

that both purifying selection and activity rate evolution can affect TE loads under sexual or asexual reproduction (Charlesworth and Langley, 1986; Dolgin and Charlesworth, 2006; Hickey, 1982). However, no theoretical study has considered TE load evolution under the joint effects of the different processes. To fill this gap, we extended the individual-level simulation program of Dolgin and Charlesworth (Dolgin and Charlesworth, 2006). This program allows to study the evolution of TE copy numbers in an asexual lineage as a function of TE activity (the joint effects of transposition and excision rates), as well as of the strength of selection against TE insertions, which depends on the fitness cost per TE insertion. To compare TE loads in sexual and asexual lineages, we first extended the program to include events of sexual reproduction and parameterized the simulations with empirically determined values from yeast (Blanc and Adams, 2004; Carr et al., 2012; Garfinkel et al., 2005). We ran individual-based simulations with a range of transposition rates, excision rates and selection coefficients with and without epistasis between TE copies as pertinent for yeast (see **Supplementary file 2A**).

For all simulations, TE loads in populations undergoing sex every 90 generations decreased faster than in asexual populations, contrary to our empirical observations. This occurs because sexual events generate variation among individuals in TE loads (and thus variation in fitness), which facilitates selection against deleterious TEs (see also Dolgin and Charlesworth, 2006). Different transposition rates under meiosis (sex) or mitosis (asex) did not affect this finding. Indeed, increased TE activity during meiosis only transiently increases TE loads in sexual strains. Because such activity also generates

increased variation in TE loads (and therefore in fitness) among strains, the additional TE copies generated during meiosis are rapidly removed by purifying selection (**Figure 1-figure supplement 3**). In short, none of the simulations generated the empirically observed pattern of lower TE loads in asexual than sexual strains. In a second step, we therefore allowed TE activity rates to evolve over time, by introducing a modifier allele that increases excision rates. The allele has no direct fitness effect, so it can only be fixed in a population via genetic hitchhiking. In simulations that included the modifier allele, the modifier spreads rapidly to fixation in asexual strains, because it is associated with genomes that have fewer TE copies, and therefore have a higher relative fitness. As a consequence, TE activity rates decrease in asexual populations (**Figure 1-figure supplement 4**). By contrast, the modifier cannot spread as rapidly in sexual populations because recombination constantly breaks up the association between the modifier and less TE loaded backgrounds. By allowing for the evolution of TE activity rates in our simulations, we were able to identify parameter values representative for yeast that result in simulations with a very close fit to our empirical results (**Figure 1B, Supplementary file 2B**). These analyses thus corroborate our empirical findings that a likely mechanism driving genomic TE load reduction in asexual yeast strains is the rapid evolution of increased TE excision rates. A similar effect would be expected if our modifier acted on transposition rather than excision rates, since the net TE activity depends on the relative rates of transposition vs excision. However, our empirical results do not suggest major differences in transposition rates between sexual and asexual yeast strains. In combination with our findings that, in the absence of TE activity evolution,

sexual strains always lose TEs faster than asexual ones, the empirical results are best explained by an increase in TE excision rates under asexuality (**Figure 1, 2**).

Our study shows that sexual reproduction permits the maintenance of TEs in *S. cerevisiae*, while in its absence, TE loads decrease, likely via the evolution of TE activity rates. The findings are consistent with empirical findings of low TE activity in old asexuals (Bast et al., 2016) and the idea that TEs should evolve to be benign in asexual species, because the evolutionary interests of TEs and their host genome are aligned (Charlesworth and Langley, 1986). While the exact mechanisms causing TE activity change in the asexual yeast populations cannot be assessed in the empirical data, our simulations suggest that there is some form of TE defense mechanism (a 'modifier locus') that either segregates in the ancestral yeast strain used in the experiments or repeatedly appeared *de novo* during experimental evolution. Independently of the exact mechanism, we confirm that TE loads do not increase, but decrease, in asexual populations. This contrasts with the hypothesis that most asexual species are evolutionarily short lived because they are driven to extinction via negative consequences of accumulating TE copies (Arkhipova and Meselson, 2005). Instead, sex is at the root of the evolutionary success of parasitic TEs.

Methods

Yeast experimental evolution

We used data generated in a previous study based on experimental evolution of the yeast *S. cerevisiae* (for in-depth details see (McDonald et al., 2016). In

short, 12 different strains were initiated from the same pool of ancestral strains (derived from haploid W303 strains) and kept under constant conditions. Sexual reproduction in yeast depends on the presence of two separate mating types. Only individuals with different mating types can fuse and go through meiosis. Asexual reproduction occurs through budding. For the experiment, six haploid strains consisting of mating type a (MATa) and six haploid strains of mating type α (MATα), were grown over 990 generations. Of these, four strains were grown exclusively asexually (two of MATa, two of MATα), while the eight others (four of MATa, four of MATα) were mixed for mating events every 90 generations, resulting in four sexual strains. Paired-end Illumina reads were generated for each of the 12 different strains every 90 generations during 990 generations (for a total of 11 sequencing events per strain). Read numbers per sample ranged from 12,775 to 10,270,312, averaging 2,964,869 reads per sample, with a total of 818,303,966 reads. Details of the read data can be found at BioProject PRJNA308843 and in the original study (McDonald et al., 2016).

Data processing

The genome of the haploid W303 *S. cerevisiae* strain was retrieved from (Lang et al., 2013). All Illumina paired-end raw reads of the 12 replicate strains generated in (McDonald et al., 2016) were downloaded from the SRA (BioProject identifier PRJNA308843). Raw reads were quality filtered by first removing adapter sequences (with the script used in the original study (McDonald et al., 2016), provided by Daniel P Rice, Harvard University), followed by removing the first 10 bases and quality trimming using trimmomatic v0.33 (Bolger et al., 2014) with parameters set to LEADING:3

TRAILING:3 HEADCROP:10 SLIDINGWINDOW:4:15 MINLEN:36. Additionally, non-overlapping paired-reads were constructed *in silico* from the subset of the original paired-reads that were overlapping, as a prerequisite to run the insertion detection pipeline. For this, overlapping reads (on average overlapping by 16 bp) were merged using PEAR v0.9.6 with standard parameters (Zhang et al., 2014). Merged reads were split in half and 20 bp deleted from each read at the overlapping ends using the fastx_toolkit v 0.0.13.2 (Hannon Laboratory, 2010). This resulted in mean read lengths of 72 bp. These 'artificial' non-overlapping read pairs were afterwards merged with the read set fraction that was non-overlapping.

Overall transposable element load

A *S. cerevisiae* specific, curated and updated TE library that contained all consensus sequences of all TE families found in this species is available from Carr et al., 2012. With this library, we identified TE content and specific copy insertions in the W303 genome using RepeatMasker v4.02 (Smit et al., 2013-2015) with parameters set to -nolow -gccalc -s -cutoff 200 -no_is -nolow -norna -gff -u -engine rmblast. For overall TE load estimates, the fraction of reads mapped to TEs out of total mappable reads was calculated. For this, the TE library was appended to the masked W303 genome and all reads for all strains and generations were mapped using BWA v0.7.13 with standard parameters (Li, 2013). For all strains, mean per-base coverage was checked with bedtools genomecov v2.26 (Quinlan and Hall, 2010), upon which the asexual strain 3D-90 was excluded from all further analyses, as coverage was lower than one-fold for this sample. Following this analysis, stat-reads from the PopoolationTE2 v1.10.04 program (Kofler et al., 2016) was utilized to

extract the number of total mapped reads and reads mapped to TEs. For statistics, a permutation ANOVA with the formula lm(coverage~generation*mode) was utilized; for details see github repository.

Specific transposable element insertions

To detect specific reference (present in the reference genome) and non-reference TE insertions in all samples, the McClintock pipeline was utilized (Nelson et al., 2017). This pipeline combines six different, benchmarked programs in a standardized fashion. McClintock was run with the non-overlapping read set,the curated TE library, and the W303 assembly using default parameters. The nonredundant insertions output file per sub-program was collected. Next, we utilized a custom python script to collect all information on insertions detected by all different programs and counted insertions with evidence from different programs only once.

To identify full-length TEs and solo LTR insertions from the McClintock custom filtered output, we tagged insertions by length according to the typical *TY* TE properties found in *S. cerevisiae* (i.e. a full TE is a combination of internal sequence and two LTRs within a 500 bp range; solo LTRs are between 220 and 420 bp; see **Supplementary file 1**). Because TE insertion detection was influenced by the coverage, coverage was taken into account when calculating the number of insertions, by adding it as random factor (coverage effect $P < 0.001$, generation effect $P = 0.006$, reproductive mode effect $P = 0.033$, and interaction between generation and mode $P < 0.001$; permutation ANOVA with the formula lm(counts~coverage+generation*mode); for details see github repository). We then calculated the number of lost TEs in asexual strains from the regression slope in asexuals after correcting for

coverage (i.e. computing residuals) over 1000 generations, with 50 full-length TEs in the ancestor. To additionally check for a bias due to coverage differences between sexual and asexual strains, we randomly subsampled read data for each sample corresponding to the mean coverage of the asexual strains for each generation (**Figure 1-figure supplement 2)**.

Modelling

To model TE dynamics in yeast we adjusted an individual based, forward in time simulator by Dolgin and Charlesworth (Dolgin and Charlesworth, 2006). We extended the model to include sexual cycles via fusion of two haploid individuals and recombination, with on average one crossover on each of the 16 modeled chromosomes (yeast has 16 chromosomes; Goffeau et al., 1996; McDonald et al., 2016). Each chromosome carries 200 loci that are potential targets for a TE insertion. A simulation is initiated with a single individual with 50 TEs randomly placed in the 3200 loci of the genome. The founder individual then populates clonally the whole simulated deme of explicitly simulated 100,000 individuals.With currently available computational resources, there was no need to scale deterministic parameters of the model as was done in the original study by Dolgin and Charlesworth (2006). To account for mutations during this phase we ran 20 burn-in generations of transposition and excision cycles on every individual separately without applying selection. One generation in the simulation consists of a round of selection and reproduction with transposition occuring during reproduction, followed by excision. The relative fitness w_n of an individual carrying n TEs was modeled as $w_n = exp(-an - \frac{1}{2}bn^2)$, where a and b are parameters representing the strength of selection and the strength of epistatic interactions

between TEs respectively (Dolgin and Charlesworth, 2006). The simulation was then continued for 990 generations. We performed 10 replicates of each simulation. Using the average TE load in the population measured every ten generations, we fitted a linear model to estimate average TE loss across the ten replicates of each simulation. Parameters were derived from yeast experimental measurements and simulations were run with perturbation in the surrounding parametric space (see **Supplementary file 2A**). We further explored the effects of different transposition rates during meiosis vs asexual reproduction, but this did not change the dynamics even for meiotic transposition rates that were not biologically plausible (up to 10% of TEs transposing during meiosis). The last extension included the introduction of an unlinked, general modifier allele increasing the excision rates of all elements by the same amount. The parameters related to this extension are the initial frequency of the modifier allele and the excision rate increases when the modifier allele is present (see **Supplementary file 2B**). See the code documentation for details.

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Competing interests

Authors declare no competing interests.

Data availability

Raw read data of the experiment is available at SRA (BioProject identifier PRJNA308843).

Code availability

The code used for both the analyses of empirical data and for the theoretical prediction of TE dynamics together with explanations are available online at https://github.com/KamilSJaron/reproductive_mode_TE_dynamics

Figure labels

Figure 1. Sex maintains constant TE loads through time, while its absence leads to TE copy number reductions, for both (**A**) empirical data and (**B**) simulations including an allele modifying TE activity rates. **(A)** Number of full-length TE copies inserted in genomes of four replicates of otherwise identical occasionally sexual (red) and wholly asexual (blue) yeast strains over 1000 generations of experimental evolution. Numbers are expressed as residuals, since the TE detection probability depends on sequencing coverage (Figure 1-figure supplement 2). **(B)** Individual-based simulations for studying the TE load dynamics expected under sexual and asexual reproduction with ten replicates (red and blue dotted lines). The simulations are parameterized with yeast-specific values and include a modifier alleles. For both (A) empirical and (B) simulation data, asexuals lost about nine active, full-length TEs by generation 1000. Lines represent linear regression for sexuals (red) and asexuals (blue) and the grey areas represent 95% CI.

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Figure 1-figure supplement 2. Identification of TE insertions depends on the sequencing coverage. (A) TE insertions (including those present in the reference genome and *de novo* insertions) *vs*. median sequencing coverage from paired reads. Coverage influences the ability to detect TE insertions (Wilcoxon signed-rank test V = 4095, p-value < 0.001). (B) Median read coverage per sample for sexual (red) and asexual (blue) strains over 1000 generations. Data from asexual strains had lower coverage, but were not different to sexuals through time (generation effect *P* = 0.096, reproductive mode effect *P* = 0.002, and interaction between generation and mode *P* = 0.588; permutation ANOVA). Lines represent linear regression and the grey areas represent 95% CI. (C) Subsampling to the mean asexual read coverage per generation for all samples results in similar findings (generation effect P = 0.012, reproductive mode effect P = 0.302, and interaction between generation and mode P = 0.004; permutation ANOVA).

Figure 1-figure supplement 3. In the simulations the spread of a modifier of excision rates is faster in asexual than sexual populations because it remains linked to genomes that have few TE copies and therefore a high relative

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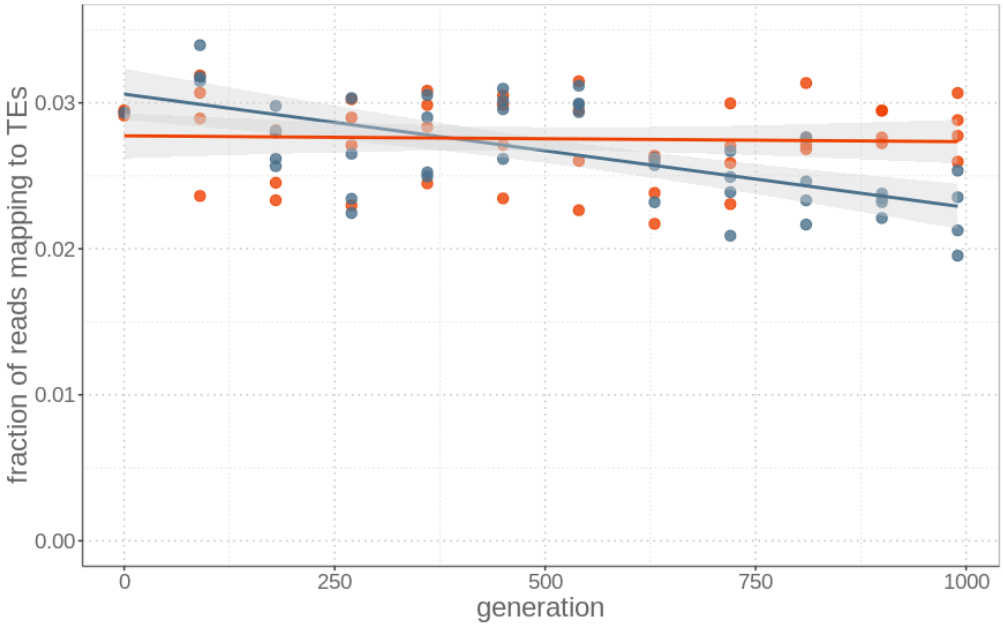

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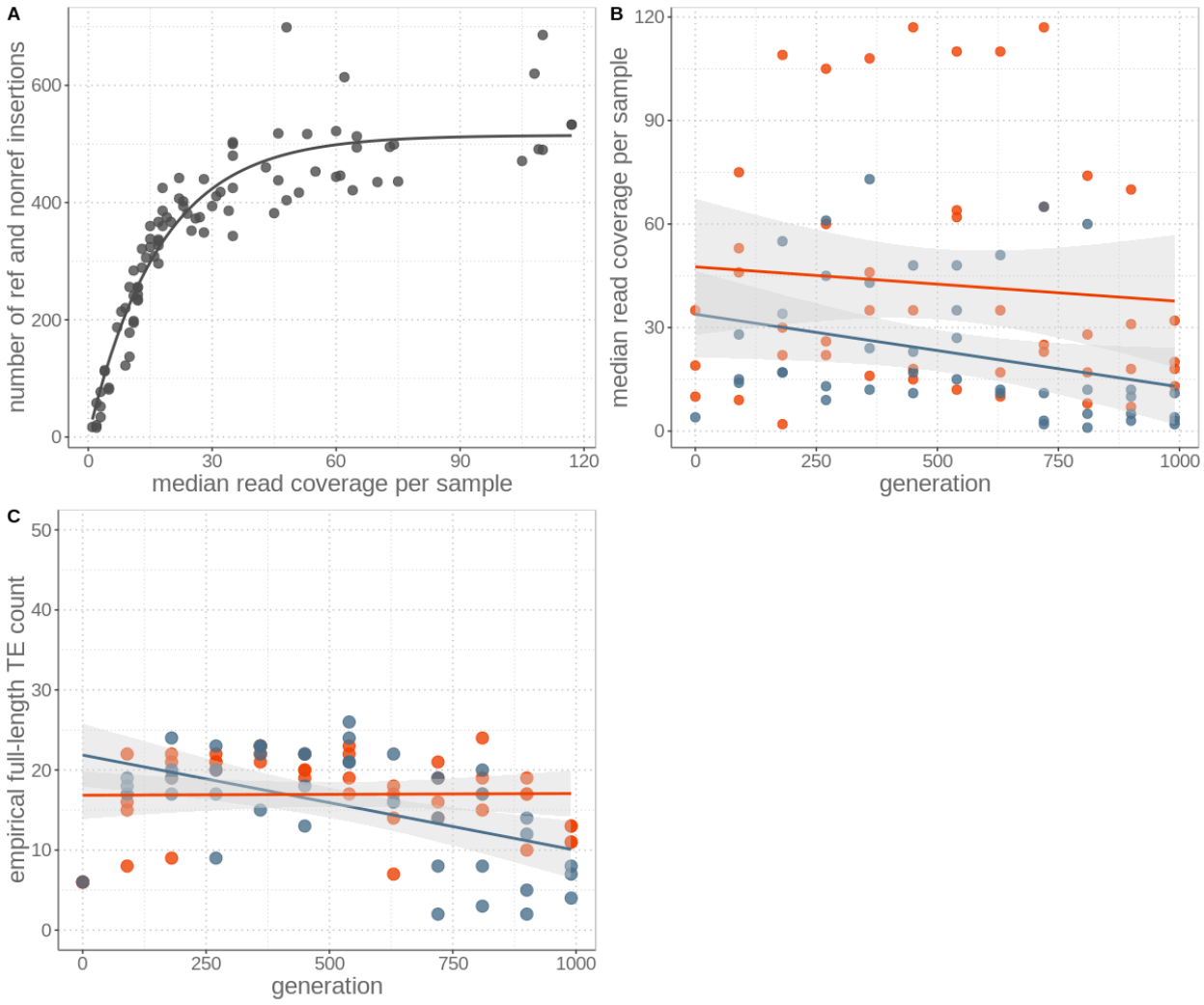

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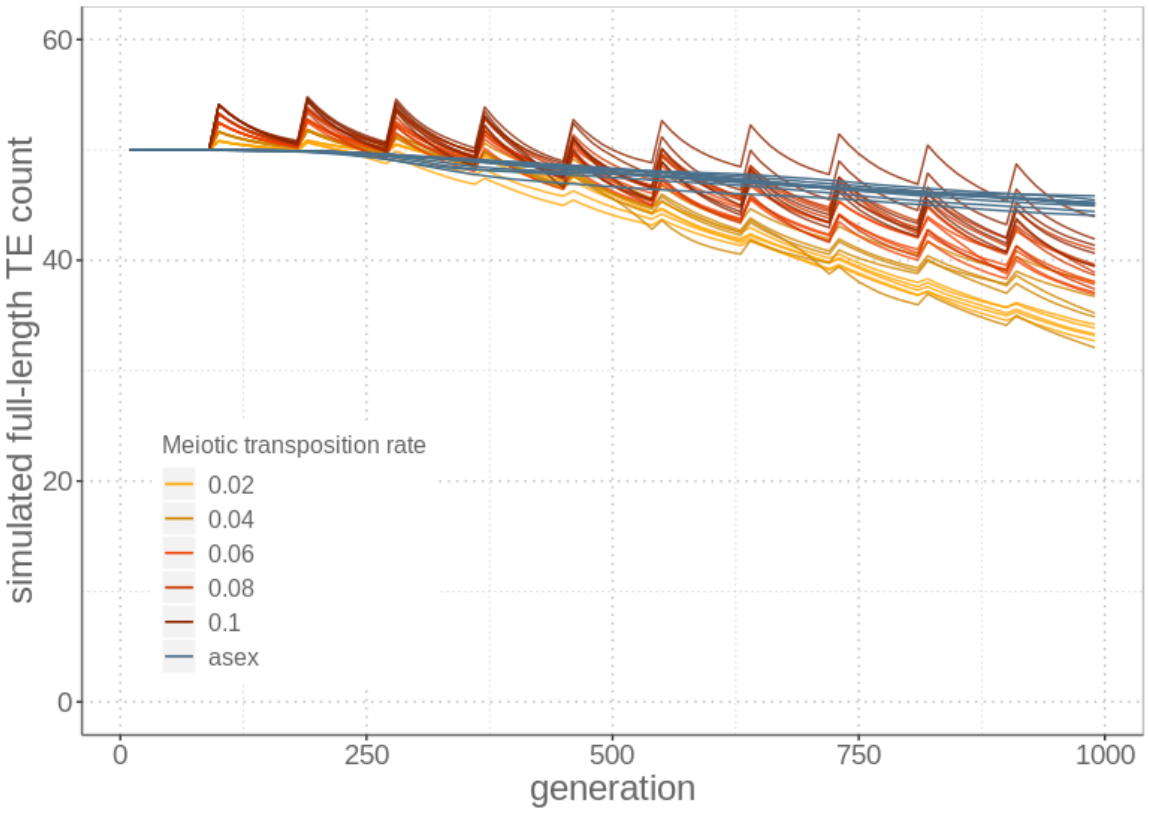

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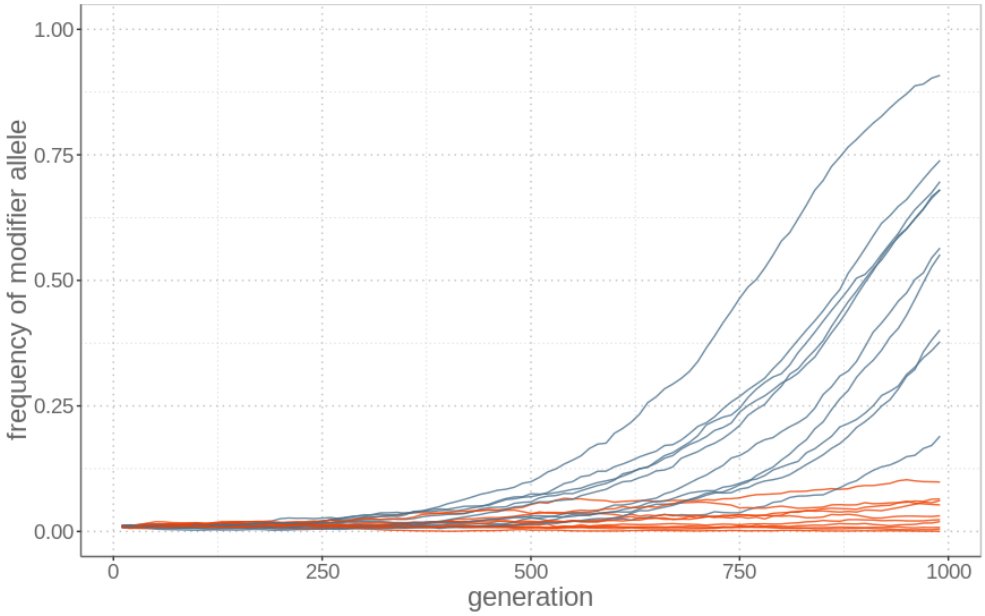

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Element	Internal		LTR	combined boundaries
range				
TY1/Copia	5249	338	5925	5425-6425
TY2/Copia	5295	332	5958	5458-6458
TY3/Gypsy	4671	340	5351	4851-5851
TY3_1p/Gypsy	4675	365	5405	4905-5905
TY4/Copia	5484	371	6226	5726-6726
TY5/Copia	4874	251	5376	4876-5876

Supplementary file 1. *S. cerevisiae* TY elements and the sizes (in bp) of internal regions and LTRs and the size boundaries used for filtering.

transposition rate	exision rate	selection a	sel. b	sex lost TEs	asex lost TEs
1.00E-06	5.00E-07	1.00E-04	0	6.5	2
1.00E-05	5.00E-07	1.00E-04	0	6.7	1.5
1.00E-04	5.00E-07	1.00E-04	0	3.5	-0.8
1.00E-06	1.00E-06	1.00E-04	0	8.1	2.2
1.00E-05	1.00E-06	1.00E-04	0	7.8	2.3
1.00E-04	1.00E-06	1.00E-04	0	5.7	-0.1
1.00E-06	5.00E-05	1.00E-04	0	28.6	17
1.00E-05	5.00E-05	1.00E-04	0	21.9	13.1
1.00E-04	5.00E-05	1.00E-04	0	19.4	11
1.00E-06	5.00E-07	5.10E-04	0	6.7	1.9
1.00E-05	5.00E-07	5.10E-04	0	6.4	1.6
1.00E-04	5.00E-07	5.10E-04	0	4.3	-0.2
1.00E-06	1.00E-06	5.10E-04	0	9.1	2.5
1.00E-05	1.00E-06	5.10E-04	0	8.2	2.4
1.00E-04	1.00E-06	5.10E-04	0	6.3	0.5
1.00E-06	5.00E-05	5.10E-04	0	22.7	14
1.00E-05	5.00E-05	5.10E-04	0	22.6	13.6
1.00E-04	5.00E-05	5.10E-04	0	20.6	11.3
1.00E-06	5.00E-07	1.00E-04	0.00039	6.5	2
1.00E-05	5.00E-07	1.00E-04	0.00039	6.7	1.5
1.00E-04	5.00E-07	1.00E-04	0.00039	3.5	-0.8
1.00E-06	1.00E-06	1.00E-04	0.00039	8.1	2.2
1.00E-05	1.00E-06	1.00E-04	0.00039	7.8	2.3
1.00E-04	1.00E-06	1.00E-04	0.00039	5.7	-0.1
1.00E-06	5.00E-05	1.00E-04	0.00039	28.6	17
1.00E-05	5.00E-05	1.00E-04	0.00039	21.9	13.1
1.00E-04	5.00E-05	1.00E-04	0.00039	19.4	11
1.00E-06	5.00E-07	5.10E-04	0.00039	6.7	1.9
1.00E-05	5.00E-07	5.10E-04	0.00039	6.4	1.6
1.00E-04	5.00E-07	5.10E-04	0.00039	4.3	-0.2
1.00E-06	1.00E-06	5.10E-04	0.00039	9.1	2.5
1.00E-05	1.00E-06	5.10E-04	0.00039	8.2	2.4
1.00E-04	1.00E-06	5.10E-04	0.00039	6.3	0.5
1.00E-06	5.00E-05	5.10E-04	0.00039	22.7	14

1.00E-05	5.00E-05	5.10E-04	0.00039	22.6	13.6
1.00E-04	5.00E-05	5.10E-04	0.00039	20.6	11.3

Supplementary file 2A. Explored parameter space of the simulations as pertinent for yeast (empirically determined values in bold). Selection_a and selection_b are selection coefficients for linear fitness effects and epistasis, respectively. Lost_TEs refers to the total number of TE lost after 1000 generations (averaged over ten replicates).

init_f	selection_a	selection_b	sex_lost_TEs	asex_lost_TEs
0.01	2.00E-04	0	0.6	1.2
0.01	3.00E-04	0	0.7	2.7
0.01	4.00E-04	0	0.6	5.3
0.01	0.000425	0	0.9	6.2
0.01	0.00045	0	0.7	6.6
0.01	0.000475	0	0.8	10.3
0.01	5.00E-04	0	1	9.9
0.01	5.00E-04	1.00E-06	0.3	0.3
0.01	1.00E-03	1.00E-06	0.6	0.9
0.01	2.00E-03	1.00E-06	1.3	5.1
0.01	2.00E-04	1.00E-05	1.3	15.6
0.01	3.00E-04	1.00E-05	1.2	17
0.01	4.00E-04	1.00E-05	2.2	20.1
0.01	5.00E-04	1.00E-05	2.2	22.7
0.1	2.00E-04	0.00E+00	3.4	7.6
0.1	3.00E-04	0.00E+00	4	12.6
0.1	4.00E-04	0.00E+00	4.9	16.9
0.1	5.00E-04	0	6.4	20
0.1	2.00E-04	1.00E-05	7.6	22.4
0.1	3.00E-04	1.00E-05	8.8	23.5
0.1	4.00E-04	1.00E-05	10.6	24.9
0.1	5.00E-04	1.00E-05	11.9	26.1

Supplementary file 2B. Explored parameter space for simulations including a modifier allele. Highlighted is the simulation closest to empirical observations. Init_f is the frequency of the modifier at the start of the simulations. Selection_a and selection_b are selection coefficients for linear fitness effects and epistasis, respectively. Lost_TEs refers to the total number of TE lost after 1000 generations (averaged over ten replicates). The bold lines refer to parameter combinations that generate results close to the observed empirical values.

Chapter 5

Discussion

Kamil S. Jaron

Chapter 5: Discussion

Sex is a prevalent trait shared by nearly all eukaryotes. As there are only a handful of lineages that lost sexual reproduction, understanding the role of sex and recombination is one of the central questions of evolutionary biology[1]. There are many studies suggesting general explanations for the prevalence of sex, but practically all of them are contrasting sexual reproduction to clonal reproduction (mitotic asexuality) and assume an origin of asexuality via mutation. While this kind of asexuality is practical to model in a theoretical context, it does not reflect very well the diversity of asexual species we find in nature.

Furthermore, theory predicts extensive consequences of asexuality for genome evolution. However, the genomic properties of the majority of asexual animals I analysed in this thesis are within the ranges of what is observed in sexual genomes and not even a single genomic feature is shared by all asexuals (chapter 2). The absence of any common genomic feature among asexual species suggests that treating clonality as a single alternative to sexual reproduction might be an oversimplification. We presented the very first catalogue of genomic features in asexual genomes of multiple taxa using a single analysis framework. Although the catalogue brought many new insights, we were not able to disentangle lineage-specific evolution and consequences of asexuality by using only asexual genomes. However, we have addressed two hypotheses that were suggested based on observations in the genome of the bdelloid rotifer *A. vaga* (Flot et al., 2014). First, the large amount of horizontal gene transfer found in bdelloid rotifers (Nowell et al., 2018) seems to be a rotifer-specific feature, there is no evidence in any of the other asexual species for extensive gain of horizontally transfered genes after the transition to asexuality. Second, genomic palindromes identified in the genome of the bdelloid rotifer *A. vaga* were proposed as a mechanism to avoid mutational meltdown (Flot et al., 2014). These palindromes were not,

[1] There are approximately 10,700 studies indexed by Google scholar with exact phrase “Why sex” and 257 with this phrase in the title.

however, identified in high frequency in any asexual genome other than in *A. vaga* (Chapter 2), nor in other genomes of bdelloid rotifers (Nowell et al., 2018).

The consequences of asexuality are more apparent when contrasted to sexual sister species. In chapter 3 we compared genomes of five asexual *Timema* stick insects with genomes of their sexual sister species. Asexual species of *Timema* were previously shown to retain heterozygosity between generations (Schwander & Crespi, 2009). We found that these heterozygous loci represent only a small fraction of the genomes, and that the genomes of asexual *Timema* are largely homozygous. The low levels of heterozygosity are remarkably similar between the five asexual species. We were able to use heterozygous loci to understand the mechanism behind the loss of heterozygosity in asexual *Timema* genomes. First, we identified structural variants shared by multiple individuals in populations, suggesting that these variants are not *de novo* mutations, but are probably maintained in the population. Further we found that the heterozygous variants shared by at least two individuals are dispersed across the whole genome rather than clustered, suggesting an ameiotic mechanism behind heterozygosity loss.

In the following sections I will compare specific empirical observations with predictions presented in Chapter 1 and discuss possible explanations of the low convergence of the results with the predictions. Later in the text, I will make an attempt to consider other taxa than animals. Finally, I will present a novel hypothesis addressing the heterozygosity pattern identified in chapter 2 and discuss which species are of a special interest to further progress our understanding of genomic consequences of asexuality.

Challenging theoretical models

Most theoretical and verbal arguments predict a strong impact of the transition to asexuality on genome evolution. In this light is perhaps surprising to see that the majority of asexual genomes we have sequenced till now do not show very unusual genomic features. Here I will follow the structure of the first

chapter and comment on each prediction, given the data presented in this thesis and previous studies. I have decided to comment on all the predictions for completeness, although the empirical data presented do not bring many new insights to all of them.

Reduced efficiency of natural selection

The prediction regarding the efficacy of selection is perhaps the strongest prediction for asexual genome evolution. Reduced efficiency is expected in all finite asexual populations regardless of the origin or the cellular mechanism. Of eight genome-wide studies of mutation accumulation only two support higher mutation accumulation in asexual species, and two support the opposite (see details in Chapter 2). Results in contradiction to this prediction were rather unexpected and illustrate how the potential benefits of recombination depends on a range of parameters, notably population size, an aspect that does often not obtain sufficient attention in biological textbooks (Bell, 1982).

More efficient selection in asexual lineages compared to their sister species was detected in oribatid mites (Brandt et al., 2017). The authors suggested that asexual population sizes might exceed population sizes of their sexual sister species, which is also supported by analysis of mtDNA, which is asexual in both sexual and asexual species. Such asexual clades are therefore not predicted to go extinct in the long term as a consequence of accumulated mutation load.

Arguably, mutation accumulation is the most important prediction regarding asexuality, as it is frequently claimed to explain expected high extinction rates of asexual lineages. However, to characterize circumstances that allow asexuals to escape the prediction of less efficient selection in natural populations, we need to test this prediction in many more species. Furthermore, more testing would provide insights into the proportion of exant asexual lineages that may already be on the way to extinction.

Lack of support for the Meselson effect

Old asexual lineages are expected to accumulate mutations independently in the homologous haplotypes. This is known as the Meselson effect (Birky, 1996). Although it is more than twenty years since this idea was proposed, the only accepted empirical example is the human pathogen *Trypanosoma brucei gambiense* as it is the only species where the Meselson effect was tested and confirmed with whole genome data (Weir et al., 2016). Although an appropriate population genomics dataset for a comprehensive test of the Meselson effect is not currently available for most other asexual species, we can derive some conclusions using the individual genomes analysed in chapters 2 and 3. Species are expected to carry a substantial amount of heterozygosity if the haplotypes have been divirging for a long time and therefore elevated heterozygosities are a prerequisite for Meselson effect to be claimed. In Chapter 2 we proposed that hybrid origin is more likely the explanation for heterozygosity patterns found in asexual species. Later in this discussion, I also propose explanations and more thoughts regarding species of unknown origin in the section **Heterozygosity patterns**.

Perhaps the most parsimonious explanation of why the Meselson effect is not supported in any animal studied so far is meiotic gene conversion, and potentially other forms of genome homogenization. Mutations are infrequent, so it would take many generations to observe significant divergence between haplotypes. Therefore even rare events of mitotic gene conversion might fully counteract it (Stoeckel & Masson, 2014). The Meselson effect is a sound and clear idea, which perhaps made it so popular among studies of asexuality. Perhaps after more than two decades of empirical research without too much success to show robust cases we should focus more on other aspects of asexual evolution.

Transposon limbo

Dependent on the model and circumstances, asexuality is predicted to lead to either increased or reduced loads of transposable elements (TEs) (Arkhipova & Meselson, 2000, 2004; Bast et al., 2016; Dolgin & Charlesworth, 2006;

Hickey, 1982). However, in practically all comparisons between asexual and sexual sister lineages the differences in TE loads were very subtle, strongly constrasting with the predictions.

In Chapter 4 we showed using a previously published dataset that asexuality can directly lead to a considerable decline of transposable elements during experimental evolution. Using simulations we have also demonstrated that asexuality can generate low TE loads through the evolution of loci that are involved in defense against TEs. We observed significant differences between sexual and asexual lines within a thousand generations, a substantially shorter time than in the case of any of the presented asexual organisms (perhaps with the exception of the marbled crayfish). Given the experimental validation of theoretical predictions of declining TE loads in asexual species, it is surprising that in natural populations the most commonly observed effect of asexulity to TE dynamics is very subtle or none. How come that we observe this transposon limbo?

Perhaps the decline of TEs in asexuals occurs only in a special type of genetic background. The simulations without a general modifier in Chapter 4 predicted increased TE load in asexuals, even when we allowed different transposition rates during mitosis and meiosis. Maybe the asexual species analysed do not vary in transposition rates among transposons, or variability in the defense mechanisms. When an association of low TE load and benign TEs cannot evolve, we should not expect TE loads to decline under asexuality. Even so, TE reductions in specific genomic background only do not explain why there are no observed cases of increased transposition activity asexual sister species as expected in the absence of the modifier allele.

In the discussion of chapter 3, I suggested one potential reson why we do not observe a decline of TE loads in asexual species. I speculated that if active transposons were lethal for asexual lineages (as suggested in Arkhipova & Meselson, 2000), we should observe only lineages emerging from sexual backgrounds with low TE activities in nature. This is supported both by the

similar loads detected in sexual and asexual species (reviewed in chapter 3), and by overall low TE loads in all but one asexual animal. Under this hypothesis we expect that sexual genera with one or more asexual lineages will show lower TE activity compared to genera without any asexual lineage. However, as TE activity is related to many other lineage-specific factors, this hypothesis should be tested using closely related genera with and without asexual lineages, and probably requiring very large sample sizes, as both transposon bursts and transitions to asexuality are stochastic processes.

Resolved intragenomic conflicts

Transposable elements have been the subject of many discussions and studies, including all three chapters of this thesis. The switch of transposable element dynamics, due to the “aligned interests” of transposable elements and their hosts, has attracted a lot of attention of researchers. Selfish copy-paste transposition no longer increases the transmission rate of the transposable element in asexuals, and therefore the reduction of fitness of the host directly affects the transposable element to the same extent as the rest of the genome. “Aligned interests” is just a different phrasing for “the conflict between transposable elements and the host genome is resolved” as intragenomic conflict is generated by a mismatch of interests of different genomic elements (Burt & Trivers, 2006; Gardner & Úbeda, 2017). The absence of a intragenomic conflict upon transitions to asexuality is also the case for many other types of conflicts present in sexual species.

Intragenomic conflict has been proposed as an explanation of the origin of asexuality in endosymbiont induced asexuality (Stouthamer, Russell, Vavre, & Nunney, 2010) and recombination itself was even proposed to be a consequence of intragenomic conflict (Archetti, 2010). However neither of these two studies discusses intragenomic conflicts after transition to asexuality.

Abundance of sex vs survival of asex

The models presented in Chapter 1 mostly aim to explain the benefits of sexual reproduction, to help explain why sexual reproduction is the most prevalent reproductive mode among eukaryotes. In this context, the comparison of the models with observed asexual lineages is not entirely appropriate, as all of the asexual lineages observed are exant lineages that have not (yet?) gone extinct. Thus it is perhaps not surprising that lineages that have survived do not agree with models predicting their extinction. Therefore we need to separate questions regarding the abundance of sex (why obligate asexuals are not abundant) and questions regarding the survival of relatively old asexual lineages ("ancient asexual scandals" sensu Judson & Normark, 1996). The questions regarding the dominance of sexual reproduction will remain difficult to study, as the only option for testing direct consequences of transitions to asexuality are experimental evolution studies (such as the one described in Chapter 4). This means that these questions are difficult or even impossible to test on a broad range of taxa. On the other hand, our understanding of the survival of old asexual lineages can be very rapidly progressed with more genomic data and theoretical work that takes into consideration important factors found in nature, such as hybrid origin or gene conversion. I will discuss below the taxonomic distribution of asexuality and where further sampling would be most beneficial.

Asexuality outside of animals

If our goal is to understand the general consequences of asexuality, we should not focus only on the animal cases. Sex probably evolved in the common ancestors of eukaryotes (Cavalier-smith, 2002) and animals represent only a very small fraction of their diversity. However, the unresolved questions regarding asexuality are very different in plants and animals. For example, a large body of asexual literature addresses the handful of peculiar cases of "ancient asexual scandals", old asexual lineages that have survived for extensive periods of time - all of them being animals (reviewed in Judson & Normark, 1996; Schurko, Neiman, & Logsdon, 2009). With the exception of *Houttuynia,* all asexual plants have a close sexual relative (Whitton, Sears,

Baack, & Otto, 2008). In the following section I will describe the major differences between plants and animals and how they affect the predictions I listed in the previous section, in order to identify predictions that should hold in both taxa. Later I will also address non-plant, non-animal eukaryotes. Although there is comparatively very little research about fundamental processes in these taxa, they provide a valuable contribution to the general discussion about asexuality.

Asexuality in plants compared to animals

The plant and animal terminology regarding asexual reproduction differes in many ways (addressed in Neiman, Sharbel, & Schwander, 2014; Van Dijk, 2009). For example, in the plant literature “apomixis” (literally translated as "away from mixing") refers to asexuality via unfertilized seeds, whereas, in the animal literature, “apomixis” refers to a type of asexuality without meiosis and without recombination (mixing) between haplotypes (mitotic asexuality) (van Dijk, 2009). In this section I will respect the plant terminology, although it will be inconsistent with the rest of this thesis, which utilizes animal vocabulary.

Asexuality in plants takes two forms, vegetative reproduction (budding or sprouting) and apomixis, asexual reproduction via unfertilized seeds. Almost all plants have a capacity for vegetative reproduction (Van Dijk, 2009). However, vegetative reproduction does not include a single-cell stage and does not imply a loss of sexual reproduction. Therefore I will further discuss only apomixis (*sensu plantae*). Apomixis, just like in animals, can be achieved via mitotic division, or by an altered meiotic division generating unreduced gametes. In this sense, the prediction regarding changes of heterozygosity remains identical to the prediction in animals. The major difference from animals is that most apomictic plants are hermaphrodites and still produce functional pollen that frequently fertilizes closely related sexual individuals and can thus initiate new asexual lineages (Van Dijk, 2009). The genomic backgrounds in such a species complex are a mosaic of independently derived sexual genotypes together with the only truly asexual locus that actually causes asexuality (Grimanelli et al., 1998; Van Dijk, de Jong,

Vijverberg, & Biere, 2009). Some animals also produce males that transmit asexuality-causing alleles to sexual sister species, such “contagious asexuality” is found in aphids (Simon, Stoeckel, & Tagu, 2010), water fleas, and the parasitoid wasp *Lysiphlebus fabarum;* (Engelstädter, Sandrock, & Vorburger, 2011). However they represent a very small fraction of asexual animals. Furthermore, apomictic plants are very closely associated with polyploidy, which (unlike in animals) might have a causative link (Otto & Whitton, 2000).

Due to frequent recruitment of genetic material from sexual sister species, plants are not useful for studying the long term consequences of asexuality. However, similarly to studies in *Daphnia*, plants could be used to study the between-generation consequences of asexuality in experimental evolution. Such studies have the potential to reveal mechnisms that could be masked in old asexual lineages. Even in our work, the homozygosity pattern in asexual *Timema* was intepreted with the help of directly observed gene conversion in *Daphnia* (Flynn, Caldas, Cristescu, & Clark, 2017; Keith et al., 2016; Xu, Omilian, & Cristescu, 2011).

In angiosperms, the pollen fertilizes both the ovule and the nutritive tissue (endosperm). Healthy development of endosperm depends on a 2:1 ratio of maternal vs paternal genomes. This brings an additional constraint to transitions of plants to apomixis in addition the constraints mentioned for animals (reviewed in Neiman et al., 2014). Although there are examples of spontaneous endosperm development, most apomicts are pseudogamous - depending on pollen that fertilizes the endosperm, but does not contribute genetic information to the next generation. This is similar, but not exactly the same as pseudogamy in animals, where a sperm is needed for initiation of the egg development.

It has been argued that plant gametes are more prone to mutation accumulation than animals because the plant germ line is derived from somatic cell lines and thus more likely to incorporate somatic mutations (Van Dijk, 2009). This idea is, however, challenged by recent studies of somatic

mutations in plants that suggest a possible separation of somatic and germ lines (Lanfear, 2018; Schmid-Siegert et al., 2017; Wang et al., 2019).

Apomixis has been described in approximately 400 plant taxa (Bicknell & Koltunow, 2004) and it has been estimated that it represents ~0.01% of angiosperms, which is approximately 10 fold less than the estimated frequency of obligate asexuality among vertebrates (Otto & Lenormand, 2002). These numbers suggest that perhaps there are no, or only very few, apomictic plants with reproduction comparable to asexual reproduction in animals.

Asexuality in protists

Although unicellular eukaryotes represent at least four major eukaryotic taxa, they are rarely considered in studies of fundamental evolutionary processes. The phylogenetic position of species is often uncertain as is their reproductive mode. The majority of studied protists reproduce asexually, with occasional sexual cycles (Green & Noakes, 1995) and therefore it is hard to distinguish species with very rare sexual cycles from obligately asexual species. Only species with genomic features incompatible with sex are widely accepted as obligately asexual (Hofstatter & Lahr, 2019). Although this criterion is robust to false positives, it creates a circular argument for cataloging genomic features of asexual eukaryotes, because, by definition, all confirmed asexuals are extreme cases that strongly deviate from sexual sister species.

One of the genomic features that was repeatedly proposed to confirm asexuality is a loss of genes related to meiosis (Hofstatter & Lahr, 2019; Schurko & Logsdon, 2008). This approach makes several assumptions that we know are frequently violated in nature. The loss of genes associated with meiosis is possible only in species that reproduce without meiosis, which in not the case for many obligately asexual animal species. Another problematic assumption is that the genes involved in meiosis have functions only in meiosis. Many of these genes are likely pleiotropic as they are frequently found in nearly all asexual animals (see Chapter 2). Loss of genes associated

with meiosis is a good indication of obligate asexuality, but their presence does not indicate occasional sexual reproduction.

Heterozygosity patterns

One particularly interesting asexual protist is *Trypanosoma*, the only species where the “Meselson effect” was observed (Weir et al., 2016). The analysis used to test for the Meselson effect in *Trypanosoma* cannot disentangle a potential hybrid origin from the Meselson effect, as both hybrid origin and a Meselson effect generate congruent phylogenies of phased haplotypes (Birky, 1996; Szitenberg et al., 2017). Indeed, in all the species where a putative Meselson effect was claimed, it was later shown to be generated by an alternative mechanism, either from including paralogs instead of alleles of the same locus (Welch, Welch, & Meselson, 2008) or hybridization events (Lunt, 2008). In the *Trypanosoma* case, whole genome sequencing was utilized, therefore the paralogy explanation is unlikely. The hybrid origin of *Trypanosoma* seems also unlikely as the study included several closely related sexual strains. However it is still possible that none of the sexual parental species has been sequenced.

Some of the species analysed in Chapter 2 are highly heterozygous, although not all of these species are apomictic (Figure 5.1). However, all of these species are of hybrid or unknown origins. On the other hand, all ten known non-hybrid species feature extremely low heterozygosity levels, including old lineages like *Timema* stick insects that reproduce via apomixis. Therefore I suggest that it is the hybrid origin that explains patterns of heterozygosity in asexual species, rather than mutation accumulation between non-recombining haplotypes.

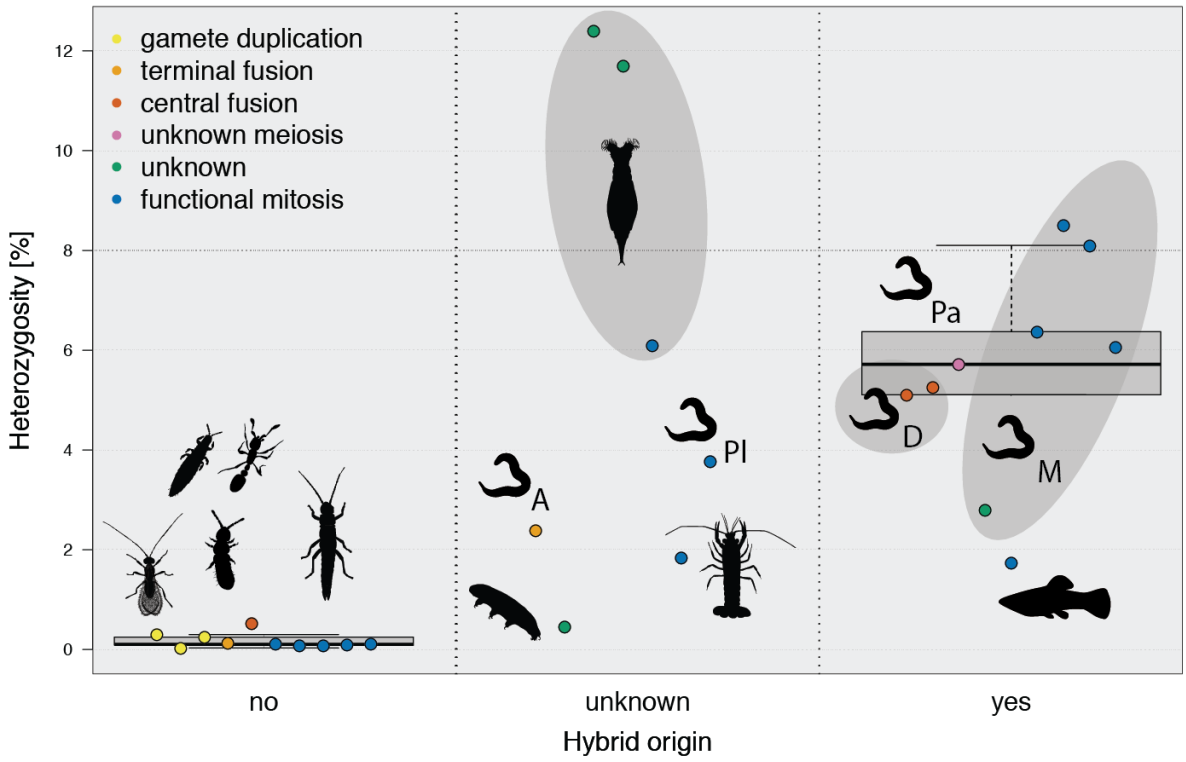

Figure 5.1: Genome-wide heterozygosity in asexual animals. Data from Figure 2, Chapter 2 were combined with additional data from Figure 1c, Chapter 3 (*Timema* stick insect heterozygosity estimates).

The association of hybrid origin and high initial heterozygosity is trivial, however any subsequent changes in heterozygosity levels are expected to depend on the cellular mechanism of asexuality. Gamete duplication and terminal fusion automixis are expected reduce heterozygosity to negligible levels in a single generation, whereas central fusion automixis is expected to gradually decrease heterozygosity depending on recombination rates (Cellular mechanisms of asexuality are reviewed in Chapter 1). Only in apomictic (mitotic) asexuals is heterozygosity expected to increase over time, and only if mutation rates are higher than rates of mitotic gene conversion. These predictions are incompatible with the observation of automictic species with high heterozygosity. First, automixis must involve central fusion, but even then the recombination rates of the species must be negligible, perhaps due to hybrid origins. Either the homologous chromosomes were already diverged enough to suppress recombination or low recombination rates were selected

to retain the heterosis. This phenomena was observed in sexual hybrids, for example in grass *Avena barbata* where early (F2) hybrids display hybrid vigor, while subsequent backcrosses (F6) suffer hybrid breakdown (Johansen-Morris & Latta, 2006). Assuming a similar fitness landscape acting in hybrid asexuals, the least recombined individuals would be the fittest and therefore low recombination rates would be favoured (Figure 5.2).

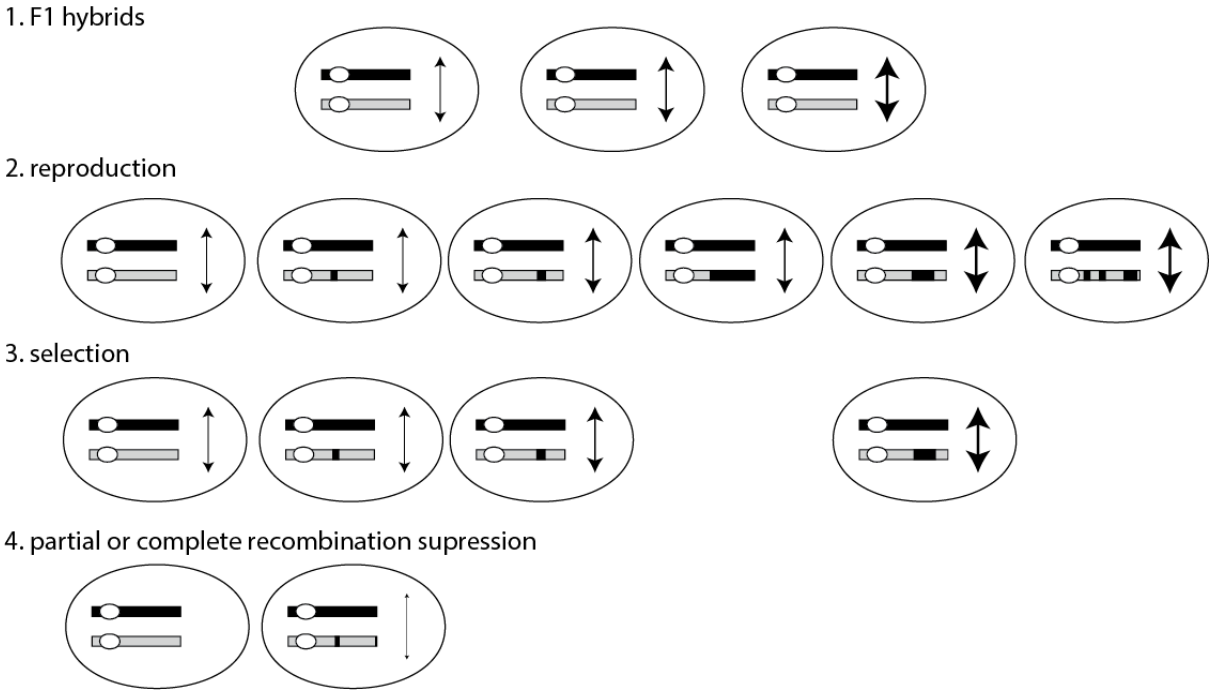

Figure 5.2: Maintenance of heterozygosity and recombination suppression as a result of selection. Assuming that F1 hybrid genotype (1.) is substantially more fit than recombinant genotypes suffering from genetic incompatibilities. Genotypes with lower recombination rates (denoted by narrower arrows) will generate less recombined perhaps even completely non-recombinant offspring (2.). As lower recombination rates are directly associated with higher heterozygosity, these individuals are favoured by natural selection (3.). If we consider recombination rates as an evolable trait, we expect that eventually, after several cycles of reproduction (2.) and selection (3.) recombination will be heavily or completely suppressed (4.).

The hypothesis of recombination suppression in hybrids can be directly tested by more genomic data from meiotic asexuals of hybrid and non-hybrid origin. The hypothesis is based on only four independently derived cases of hybrid asexuality and ten cases of non-hybrid asexuality (Figure 5.1). However, the

greatest drawback of the current dataset is the unbalanced phylogenetic representation. The confirmed asexuals of hybrid origin are nematodes and a vertebrate, while the non-hybrids are all arthropods. The only heterozygous arthropod in the dataset is the crayfish *P. virginalis*, a triploid species that was likely bred by hobby aquarists in Germany (Gutekunst et al., 2018), which, as I argue below, is also of hybrid origin.

Species of special interest should fill the taxonomic gaps with respect to the origin of asexuality, i.e.,arthropods of hybrid origin, or non-hybrid nematodes. Good candidates could be the grasshopper *Warramaba virgo*, a diploid asexual insect of hybrid origin reproducing via endoduplication (Kearney, 2003; Webb, White, Contreras, & Cheney, 1978) or *Sipyloidea similis* stick insects that appear to be both of hybrid origin and polyploid (John, Rentz, & Contreras, 1987; Kearney, 2003). In grasshoppers, high heterozygosity was suggested by variation of allozymes (Honeycutt & Wilkinson, 1989), but has not yet been confirmed on a whole genome level. Finally, an invaluable sample will be the non-hybrid, triploid, asexual snail *Potamopyrgus antipodarum*. The genome has been sequenced, but its assembly appears to be challenging, due to recent polyploidization event that pre-dated asexuality (Logsdon et al., 2017).

Origin of marbled crayfish

Previously it has been suggested that the asexual crayfish *Procambarus virginalis* is a linage derived solely from sexual *P. fallax* (Martin, Kohlmann, & Scholtz, 2007) and therefore it is autopolyploid rather than allopolyploid. The main arguments for autopolyploidy are that *P. virginalis* and *P. fallax* are morphologically very similar and that *P. virginalis* does not carry any trait of any other closely related crayfish. I believe that the crayfish is a victim of semantics, and that it is a case of hybridization between diverged lineages of *P. fallax.* However, instead of asking whether *P. virginalis* is of hybrid origin or not, I will ask whether it originated via endoduplication or fertilization of an unreduced gamete. The estimate of heterozygosity in the asexual triploid *P. virginalis* is ~1.8% (Figure 6.4a), meaning that, if endoduplication has

occurred, the divergence should correspond to the third haplotype that is diverged from the two identical copies. If so, we expect 1.8% to also be the heterozygosity of diploid *P. fallax* individuals. The heterozygosity of *P. fallax* is, however, much lower (~0.76%). This suggests that at least one of the haplotypes was acquired from a more diverged population, perhaps from a distant *P. fallax* population.

The results I show here are merely a weak indication of hybrid origin, rather than a robust proof. To conclusively determine the origin of *P. fallax*, we would need to better understand the genetic diversity of *P. fallax* and haplotype structures in *P. virginalis*, which might be problematic to explore in the near future as its big genome size (3.5Gbp) constrain makes population genomic studies difficult.

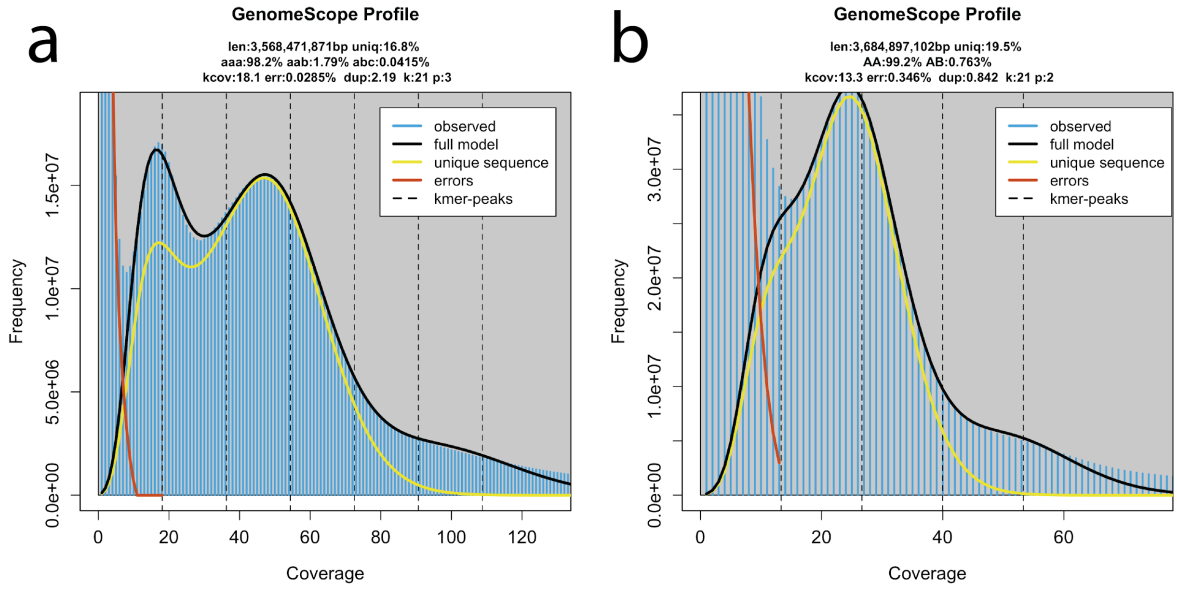

Figure 6.4: Genome profiles of marbled crayfish. **a** The triploid asexual species *Procambarus virginalis* analysed in Chapter 2 and in Figure 5.1. The estimated heterozygosity is 1.79%. **b** The diploid sexual sister species *Procambarus fallax* with an estimated heterozygosity of 0.76%. The quality of fit is less conclusive, as the error peak (red) and haploid peak (leftmost black) largely overlap.

Closing remarks

In this thesis I presented the most comprehensive empirical evidence of the consequences of asexuality on genome evolution. Although we do observe genomic consequences, they are less radical than expected from theoretical predictions and, furthermore, the consequences differ between different asexuals. I identified hybrid origin as the main reason for high heterozygosity, with little or no impact of the cellular mechanism underlying asexuality. *Timema* stick insects include five examples of homozygous apomictic species and two *Diploscapter* and *Panagrolaimus* are three examples of automictic species retaining high levels of heterozygosity. Furthermore, I presented the first study that shows that genome wide heterozygosity loss most likely occurs via ameiotic mechanisms.

I believe that progress could be made with systematic cataloging of asexual species. The work presented in chapter 2 brought many insights, although we did not choose or sequence any of these genomes. In the discussion I suggested species that should be studied next to increase the taxonomic and reproduction mode diversity of the dataset.

The main reason I advocate cataloging is the big gap between theoretical predictions and the observations based on genomic data. Knowing ranges and mechanisms acting in asexual species will provide stronger ground for building up new hypotheses and quantitative predictions, informed by biological reality. I believe that breaching the gap between theory and genomic data will be the major challenge in the field for future years.

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