Genomic features of asexual animals

Kamil S. Jaron[1, 2, †], Jens Bast[1, †], Reuben W. Nowell[3, ‡], T. Rhyker Ranallo-Benavidez[4], Marc Robinson-Rechavi[1, 2] & Tanja Schwander[1]

[1]Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland

[2]Swiss Institute of Bioinformatics, Lausanne, Switzerland

[3]Department of Life Sciences, Imperial College London, Silwood Park Campus, Ascot, Berkshire, United Kingdom

[4]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA

[†]Equal contributions

[‡]Current address: Department of Zoology, University of Oxford, 11a Mansfield Rd, Oxford OX1 3SZ, UK

Corresponding Author: Correspondence to Kamil S. Jaron (kamiljaron@gmail.com)

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, or 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 the evolution of sexual animal genomes, the genome-wide absence of recombination does not appear to have had 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 survivorship 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 a few of them have succeeded as well as their sexually reproducing relatives [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 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 question of how asexuality affects genome evolution because they focused on individual asexual lineages. Yet 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 many of the original 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 [11]. 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 [12–14]. 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 [15]. 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 the cellular mechanism underlying their 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 survivorship bias of asexual lineages are the main drivers of genome features in the studied asexual animals.

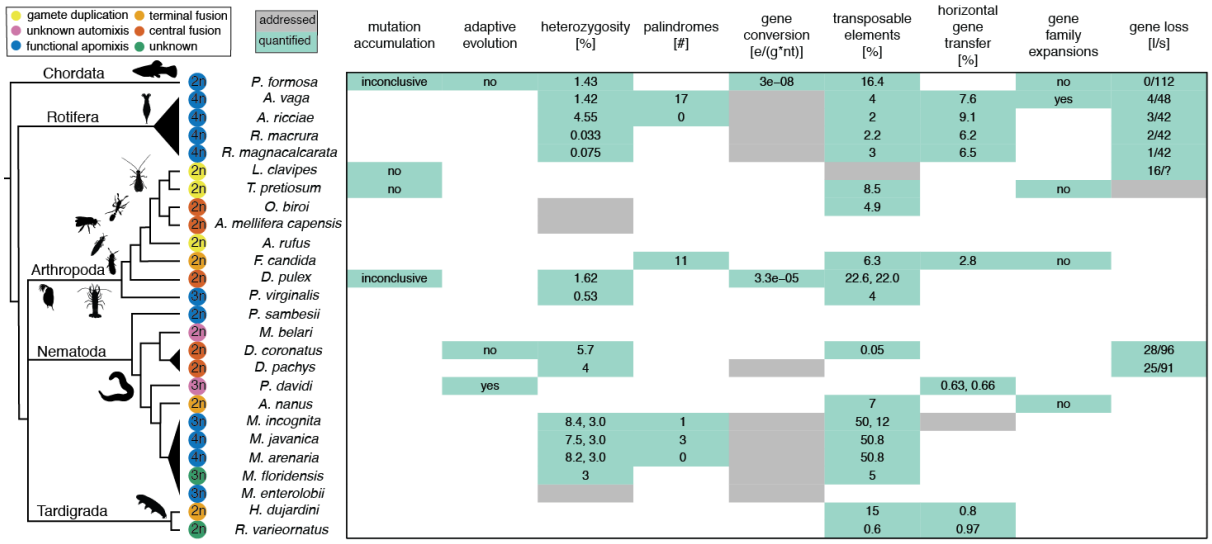


Figure 1: **Genome features studied in asexual animal species.** The phylogeny displays the taxonomic relationships of the 26 sequenced asexual animal species considered here, representing at least 18 independent transitions to asexuality. Species that might derive from the same original transition are 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 [16]. 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

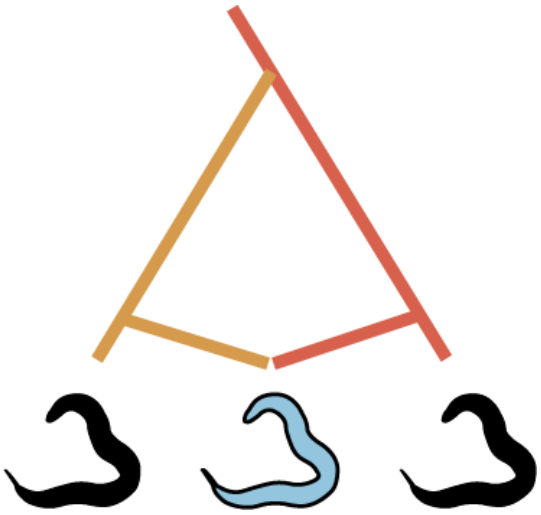
genome studies [17–40]. Findings for mutation accumulation and adaptive evolution refer to comparisons between sexual and asexual species and are reported with respect to theoretical predictions (yes: as predicted, no: opposite to predictions, inconclusive: no difference). e/(g*nt): **e**vent per **g**eneration 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 [41]. Asexual animals therefore derive from a sexual ancestor, but how transitions from sexual to asexual reproduction occur can vary and have different expected consequences for the genome [13].

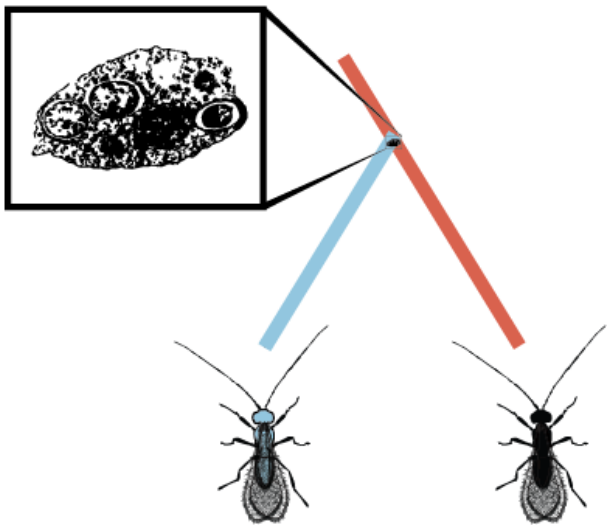
Hybrid origin:

Hybridization between sexual species can generate hybrid females that reproduce asexually [13,42]. Asexuality caused by hybridization can generate 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 [12].

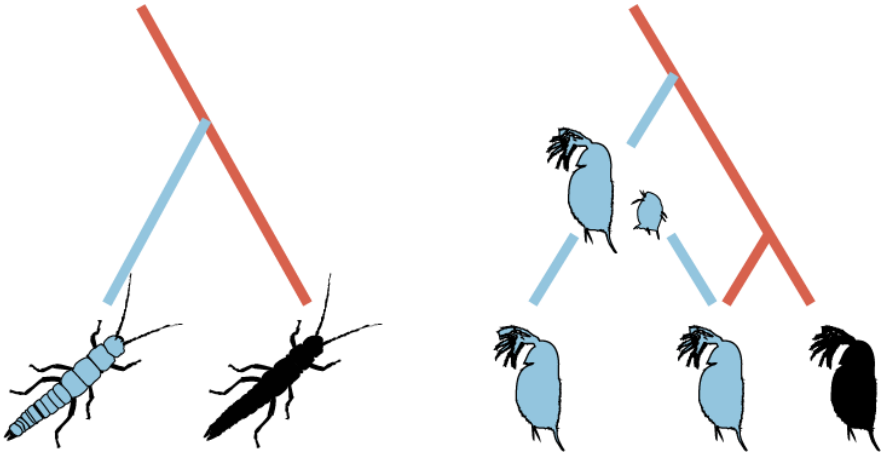


Intraspecific origins:

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 [43]. 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 [4,15].

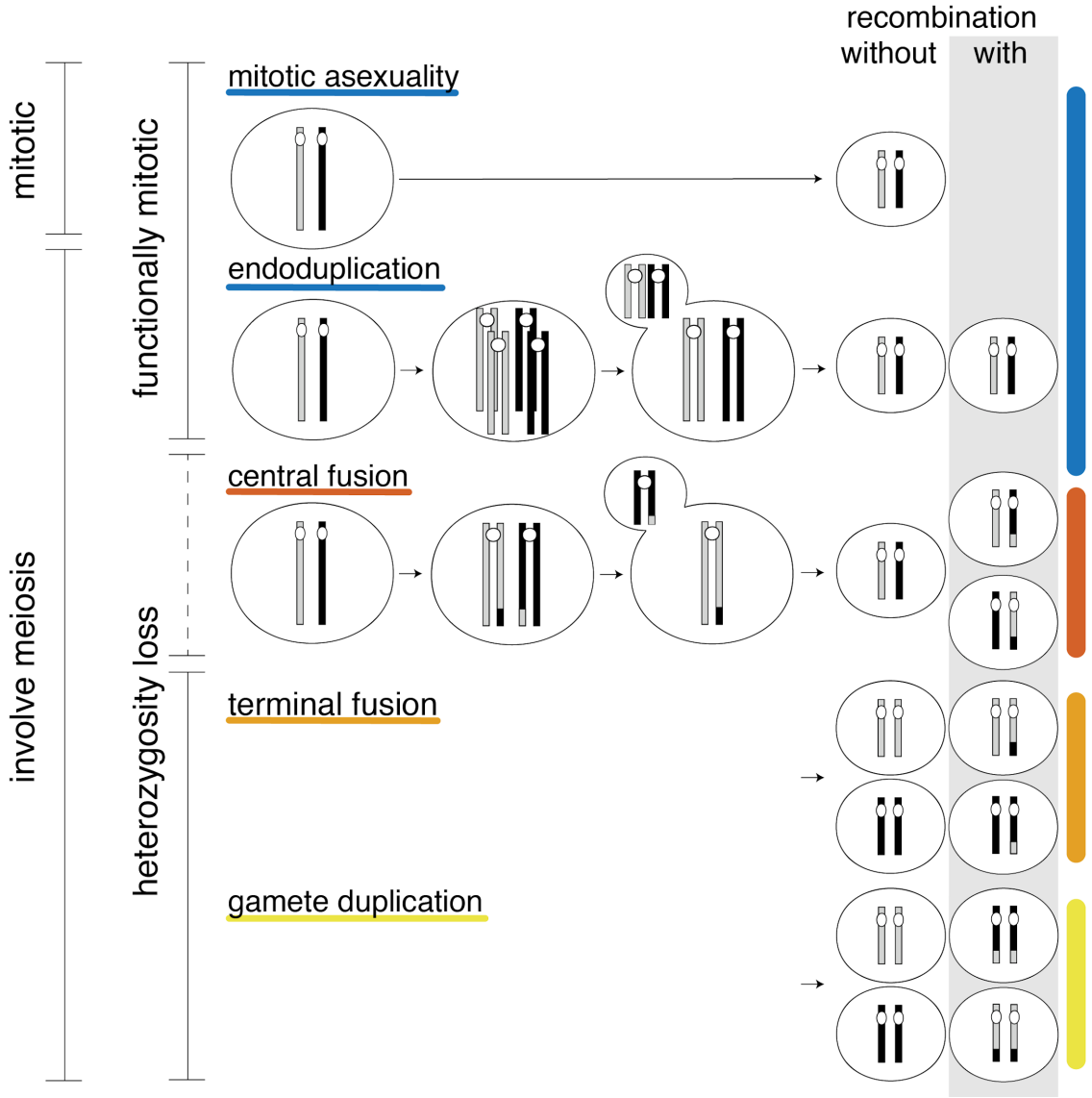
Mitotic asexuality (Apomixis). Under mitotic asexuality, no ploidy reduction occurs and offspring are clones of their mother.

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 [44] 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 24 of the 26 species. Ten of them involve (functionally) mitotic asexuality, while the 14 remaining species have different types of meiotic asexuality (**Figure 1**). All but one of the ten 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 [33] (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]**.** Therefore we did not include quantitative comparisons among asexuals with respect to their age. However, because our set of species comprises some 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 six 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 four additional studies published since [17,23,52,53]), 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 [52,54–56] of the genome-wide 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 (stick insects and

evening-primroses) [52,54]. Moreover, two studies found more deleterious mutation accumulation in *sexual* than asexual taxa (parasitoid wasps and oribatid mites) [21,55], while the four remaining ones found no differences between sexual and asexual taxa [17,23,27,56]. 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 [27].

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 homologous gene sets from 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 [17]. 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, the level of

intragenomic heterozygosity corresponds to the level of 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 of 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 [58]). 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 [16], except for bdelloid rotifers where the heterozygosity levels between homoeologs

exceeded the range quantifiable by this method (see **Supplementary Materials S2**).

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 [15,59]. 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 [8,18]. 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.

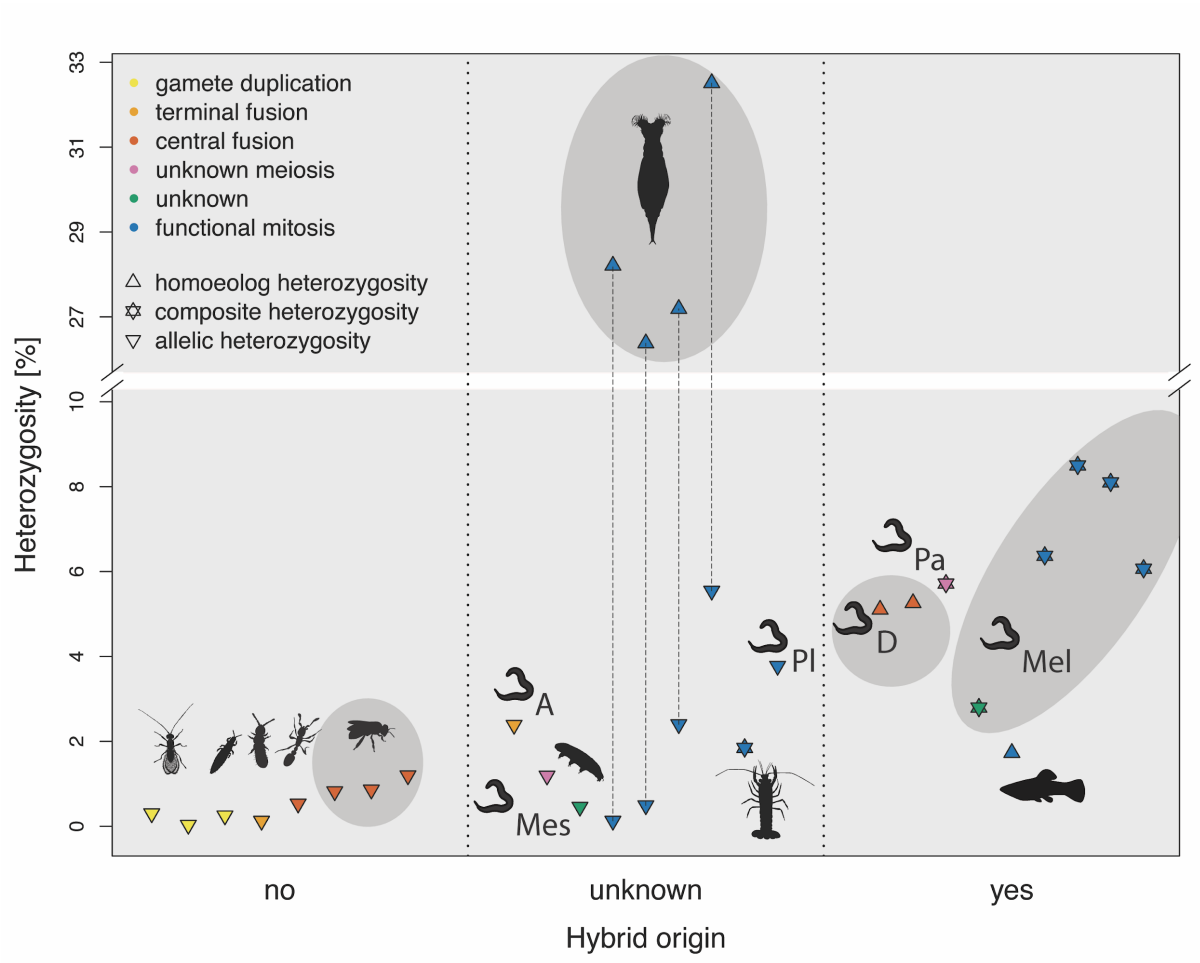


Figure 2: **Hybrid origin is the main driver of high heterozygosity in asexual species**. Heterozygosity estimates with respect to hybrid origin (x axis) and cellular mechanism of asexuality (color code). Whenever possible, heterozygosity is decomposed into heterozygosity between alleles of intra-specific 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). However, we are able to measure the haplotype structure on a per-locus basis using k-mer based approaches implemented in GenomeScope 2.0 [16]. 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 *P. virginalis* and nematode *M. floridensis*) are composed mostly of biallelic loci, while the remaining triploid species show a relatively higher fraction of triallelic heterozygosity (**Figure 3, Supplementary Materials S3**). The low proportion of triallelic loci suggests an AAB structure of the two genomes, where two of the haploid genome copies (A) are nearly identical and the last copy (B) is the carrier of the observed heterozygosity. This AAB model is in agreement with the previous genomic analysis of the asexual crayfish *P. virginalis* [30]. This previous analysis suggested that *P. virginalis* has a non-hybrid origin, which would imply that the divergence between the A and B haploid genomes corresponds to the heterozygosity present in the sexual ancestor *P. fallax* from which *P. virginalis* derived only 30 years ago. However, our estimation of the divergence of the third genome copy B (1.8%) exceeds by far our estimation of the heterozygosity in *P. fallax* (0.76%; **Supplementary Materials S4**). We therefore suggest a hybrid origin of

the crayfish B genome copy, similar to the well established hybrid origins of the other polyploid asexuals in our dataset. The second triploid species in our dataset with an AAB genome structure is the root knot nematode *M. floridensis,* which was previously mistaken for diploid (**Supplementary Materials S1**). This genome structure distinguishes *M. floridensis* from the other triploid *Meloidogyne* species (whose genomes are comprised of a larger portion with an ABC structure, **Figure 3**), which might suggest that the origin of triploidy in *M. floridensis* is independent of the origin of triploidy in the other *Meloidogyne* species.

In the tetraploid species, the biallelic loci can be sorted into those where one genome copy carries the alternative allele (yellow portions in **Figure 3**), and those where two genomic copies carry the alternative allele (pink portions). The genomes of the two tetraploid *Meloidogyne* species contain high proportions of all heterozygosity structures (**Figure 3**) suggesting a complex genomic structure, such as AABC or ABCD. Alternatively, this signal could be also caused by partial aneuploidy, which is common among *Meloidogyne* species [60].

Haplotype divergence can provide information on the origin of asexuality in polyploid species: in asexual polyploids of hybrid origin we expect, and indeed observe, highly heterogeneous divergences among haplotypes, while in those of intra-specific origin we expect homogeneous divergences. Notably, divergence between the haplotypes of the four bdelloid rotifers is highly asymmetric (**Figure 3**). 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 [61]. Studies of bdelloid rotifers traditionally refer to the divergent haplotypes as “ohnologs” (e.g., [18,19]), 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 *Adineta vaga* (2.4%) and in *A. ricciae* (5.5%). There is currently no good explanation for the higher allelic heterozygosity in *A. ricciae* compared to *A. vaga*, but kmer spectra analysis of the *A. ricciae* sequencing reads suggests that different ploidy levels in the two species could play a role (**Supplementary Materials S2**). Additionally, variable mitotic gene conversion and mutation rates could also contribute to the different allelic heterozygosities. In *A. vaga* it has been suggested that gene conversion reduces divergence between homologs in some genome regions [18] but there are currently no direct estimates for gene conversion rates in rotifers. 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 [18]). It remains possible that the subset of genomic regions with divergence between homologs in *Adineta* features 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 [62]. 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**).

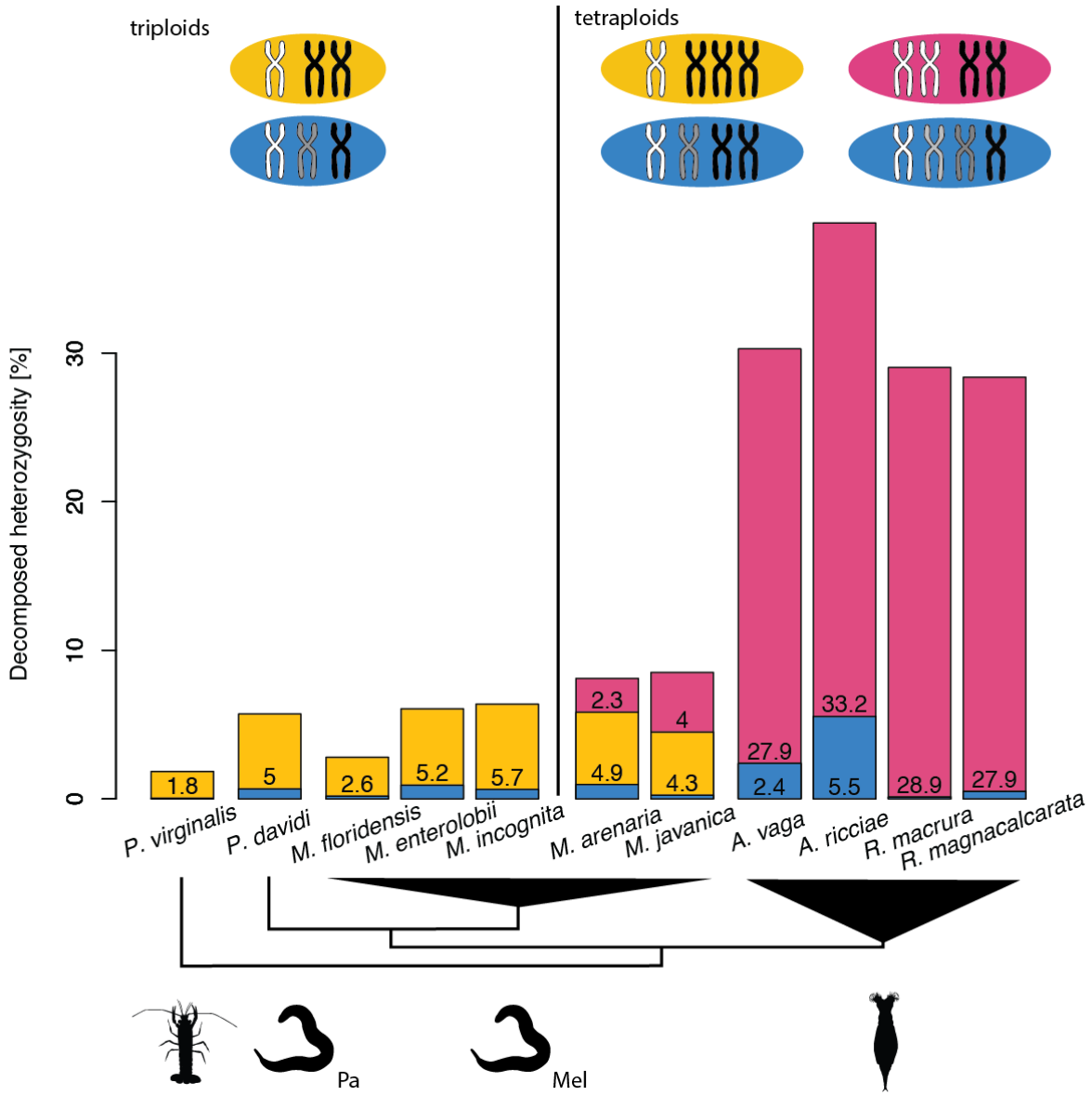


Figure 3: **The relative heterozygosity structure in polyploids.** Biallelic loci are indicated in yellow or pink: yellow when the alternative allele is carried by a single haplotype (AAB or AAAB), and pink when both alleles are represented twice (AABB). Loci with more than two alleles are indicated 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 6**). 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 [63–65]. 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 [63]. The reports of palindromes in the genomes of the bdelloid rotifer *A. vaga* [18] and of the springtail *Folsomia candida* [26] led to the hypothesis that palindromes could play a similar role in asexual organisms by 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 [64]. 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 (unlike 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 [19]). 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 Materials 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 [19]). 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 zero 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 [19] 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 [66,67].

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 Materials S5**.

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 [68,69]. 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} [27,28,70], in the amazon molly *P. formosa* to 10^{-8} [17]. Up to 11% of the genome of the nematode *D. pachys* [32] 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 [37] and rotifers [18,19], 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 [71,72]. TEs can invade genomes even though they generally provide no adaptive advantage to the individual carrying them [73–75]. 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 [75]. In sexual organisms, TEs can spread through panmictic populations because of their ability to rapidly colonize new genomes [10,76]. 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 [77,78]. 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 timescales [78,79]. Consistent with this view, initial investigations in bdelloid rotifers reported extremely low TE loads [80]. 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, even though our methods tend to underestimate TE content in bdelloids relative to other asexual species (**Supplementary Material S7**). The estimated 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%, with one species, the marbled crayfish, reaching 34.7%. The extreme repetitive content in the latter is largely inherited from its sexual ancestor, as the TE loads we estimated for the close sexual relative *P. fallax* are nearly identical (32.5%). 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%) [81]. These low abundances could stem from the evolution of reduced TE activity in asexual species [82,83], and/or appear if successful asexual taxa generally derive from sexual ancestors with largely inactive TEs and low TE contents. However, whether the apparently lower TE content in the 26 genomes is indeed linked to asexuality remains an open question as TE loads are known to be highly lineage-specific [22,38,84,85].

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)**. However, in the genomes analyzed here, we did not find any effect of cellular mechanisms on TE loads (**Supplementary Figure 7**), likely because the expected effect of the cellular mechanisms is 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 [75,86]. Similarly, we did not find any difference in TE content according to hybrid *versus* intraspecific origin of asexuals (**Supplementary Figure 7**), even though mismatches between species-specific TEs and silencing

machineries can result in increased TE activity in hybrids [12,14,87–89].

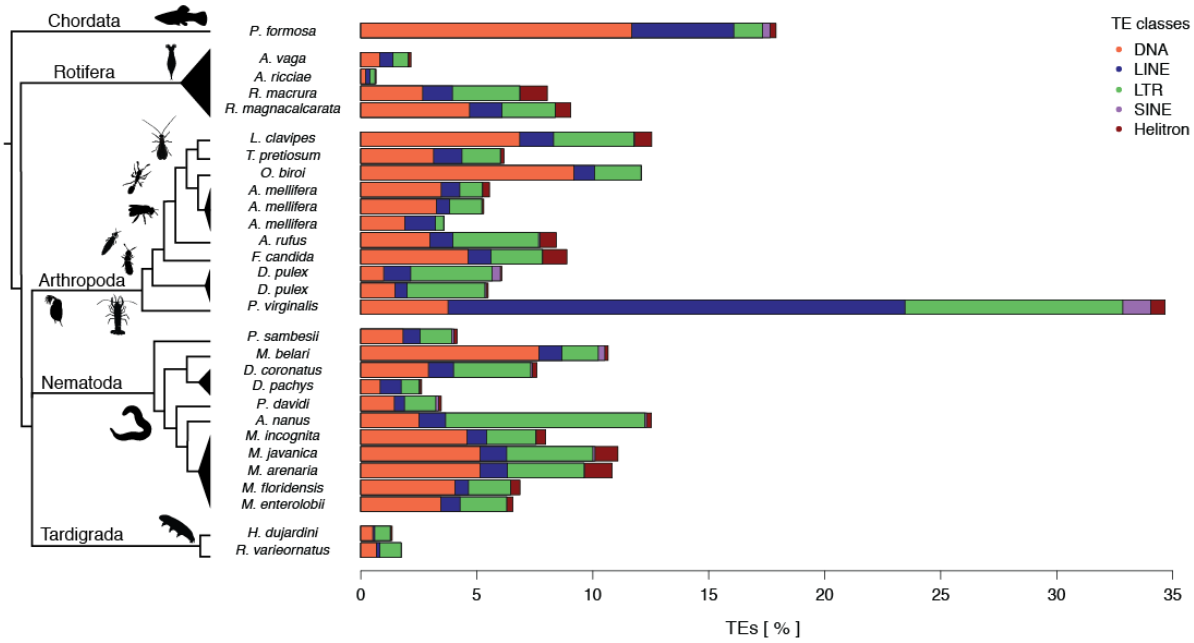


Figure 4: Percentage of transposable elements (TEs) in asexual genomes. Both the TE load and frequency of TE classes vary substantially between individual asexual lineages. The TE classes are: class I “cut-and-paste” DNA transposons (DNA), and class II “copy-and-paste” long interspersed nuclear elements 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 might represent an adaptive benefit which allows for the long-term maintenance of asexuality [90]. Indeed, bdelloid rotifers encode an unusually large proportion (6.2% - 9.1%; **Figure 1**) of horizontally acquired genes compared to sexual spiralian genomes (0.08% - 0.7%) [19,91]. Many of these have been reported to have contributed to adaptive divergence between bdelloid rotifer species [92]. 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%) [19,33,39]. The only genome with a high reported fraction of HGT (2.8%) outside of the rotifers is the springtail *F. candida* [26].

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 [19]. For each species we compared the set of annotated genes to the UniRef90 database to identify genes of likely non-metazoan origin [93]. 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).

We found no evidence for asexuality favoring the retention of HGTs. The majority of species (16 out of 23) showed a low proportion of HGT_C genes, with ~1% HGT_C (**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%) and the springtail *F. candida* (6.3%). However, we also detected unexpectedly high levels of HGT_C in the ant *O. biroi* and the wasp *T. pretiosum* (**Supplementary Table 4**). To evaluate a potential link between elevated HGT_C levels and asexuality in hexapods we additionally quantified HGT_C in a number of published genomes of sexual species from the same order or superfamily as the asexual species. This revealed a similarly high proportion of HGT_C in these sexual relatives, suggesting that whatever the cause of high HGT_C in these taxa 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 on the database used to distinguish between

metazoan and non-metazoan genes (**Supplementary Materials S6**). While our analyses show that asexuality is most likely not associated with high levels of HGT_C, the apparently high number of genes marked as HGT_C (when compared to the UniProt90 database) in hexapods requires further investigation 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 [94] that is not related to asexuality. 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 [18]. 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.

Mitotic asexuality might facilitate 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 Materials S8**). 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 [17,23,26,34]. However, only two of the four studies are based on asexuals with functionally 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) [95]. 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 [96]. 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* [31]. Furthermore, genes believed to be involved in male functions harbour an excess of deleterious mutations in the wasp *Leptopilina clavipes* [21], 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* [17].

Species reproducing via mitotic asexuality are further predicted to lose genes specific to meiotic processes [97]. The genes involved in meiosis have been studied in three of eight mitotic parthenogens (the amazon molly and the two *Adineta* bdelloid rotifers), as well as in *Rotaria* bdelloid rotifers and *Diploscapter* nematodes, whose cellular mechanisms of asexuality are unknown. There was no apparent loss of meiosis genes in the amazon molly *P. formosa* [17]. *The majority of* meiosis genes was recovered in bdelloid rotifers [18,19], and furthermore nearly all genes were also absent in the sexual species, indicating that meiosis gene loss is not associated with the evolution of asexuality [98]. *Diploscapter* nematodes also lack certain meiosis genes, but it is unknown whether these genes are also missing in sexual relatives [31,32]. 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 presumed “core” meiosis genes have also been reported in different sexual species, where meiosis is clearly functional [99].

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 they 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 potentially confounding effects of differences in assembly quality, we have utilised methods that analyse directly sequencing reads. 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" [100–102]. 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 [37,56]. 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 mitotic 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 [103,104]. 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 heterozygosity loss via meiosis and/or mitotic gene conversion plays a significant and highly underappreciated role in the evolution of asexual species of intraspecific origin and support the theoretical argumentthat one of the main benefits of sex could be the masking of recessive deleterious mutations (referred to as “complementation”) [105,106]. Conversely, high rates of heterozygosity loss could also allow for the purging of deleterious mutations, as in highly selfing species (e.g. [107,108]). 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: survivorship 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., [109,110]), 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 survivorship 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 (accessions are in **Supplementary Table 1**) and the sexual species *P. fallax* (SAMN06115719). 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* [27,35,111]). Overall, the genome features computed were: ploidy, genome size, heterozygosity, haplotype divergence structure, transposable elements/ repeat content, conserved gene content (see **Supplementary Materials S8**), 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”) [112].

We used smudgeplot v0.1.3 to estimate ploidy levels [16]. 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 [61], 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 [16]. 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 that 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 [113]. The method uses the haploid genome size (parameter -genome_size) to subsample sequencing reads to a low coverage of 0.5x (parameter -genome_coverage). We merged all trimmed sequencing reads available for each species for the input of this analysis. These subsampled reads are then assembled using an assembler (Trinity) that can deal with uneven coverages and is able to split assembled regions with few differences (including different TE families). The assembled sequences largely correspond to repetitions as non-repetitive genome regions present in the subsampled reads drop out at this stage, because the coverage of such regions is too low for assembling. 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 [113]). The output of the method is the number of sampled nucleotides assembled and annotated as different types of repeats and fractions are calculated as the numbers divided by the total number of sampled nucleotides. 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) [114], 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 Materials S5** for more details.

We assessed the impact of HGT on each asexual genome using a sequence comparison based approach, following [19]. For each species, the published set of predicted proteins were aligned to the UniRef90 (analysis presented in the main text) and UniProtKB/Swiss-Prot (**Supplementary Materials S6**) protein databases downloaded on 04/07/2019 and 14/08/2019, respectively [93,115]. The alignment was performed using DIAMOND "blastp" v0.9.21 [116] ("--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 [92]. The proportion of secondary hits that agreed with the designation (metazoan vs non-metazoan) was also recorded as the "consensus hit support" (CHS) [19,39]. 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 Materials S6**). In each case, a focal protein was designated as a putative HGT candidate (HGT_C) 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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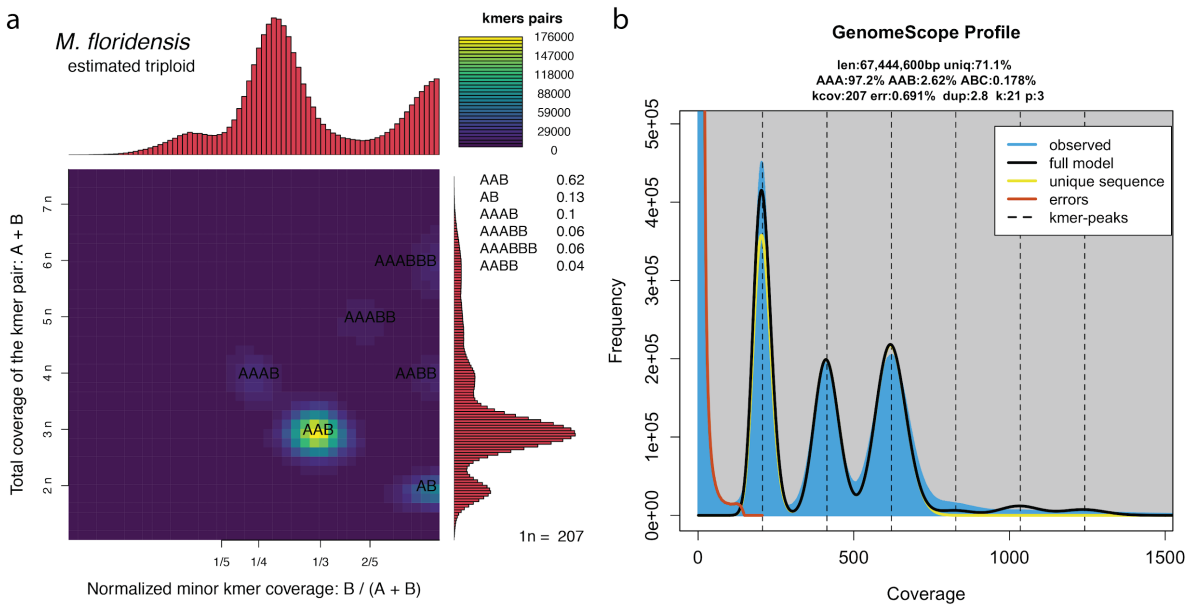
Supplementary materials

	mutation accumulation	adaptive evolution	heterozygosity [%]	palindromes [#]	gene conversion [e/(g*nt)]	transposable elements [%]	horizontal gene transfer [%]	gene family expansions	gene loss [l/s]
P. formosa	inconclusive [16]	no [16]	1.43 [16]		3e−08 [16]	16.4 [16]		no [16]	0/112 [16]
A. vaga			1.42 [17, 18]	17 [17, 18]	[17, 18]	4 [19]	7.6 [17, 18]	yes [17]	4/48 [17, 18]
A. ricciae			4.55 [18]	0 [18]	[18]	2 [18]	9.1 [18]		3/42 [18]
R. macrura			0.033 [18]		[18]	2.2 [18]	6.2 [18]		2/42 [18]
R. magnacalcarata			0.075 [18]		[18]	3 [18]	6.5 [18]		1/42 [18]
L. clavipes	no [20]					[21]			16/? [20]
T. pretiosum	no [22]					8.5 [22]		no [22]	[22]
O. biroi			[23]			4.9 [23]			
A. mellifera capensis			[24]						
A. rufus									
F. candida				11 [25]		6.3 [25]	2.8 [25]	no [25]	
D. pulex	inconclusive [26]		1.62 [26]		3.3e−05 [27, 26]	22.6, 22.0 [21, 28]			
P. virginalis			0.53 [29]			4 [29]			
P. sambesii									
M. belari									
D. coronatus		no [30]	5.7 [30]			0.05 [30]			28/96 [30]
D. pachys			4 [31]		[31]				25/91 [31]
P. davidi		yes [32]					0.63, 0.66 [32]		
A. nanus						7 [33]		no [33]	
M. incognita			8.4, 3.0 [34, 35, 36]	1 [35]	[36]	50, 12 [34, 37, 35]	[34]		
M. javanica			7.5, 3.0 [35, 36]	3 [35]	[36]	50.8 [35]			
M. arenaria			8.2, 3.0 [35, 36]	0 [35]	[36]	50.8 [35]			
M. floridensis			3 [36]		[36]	5 [37]			
M. enterolobii			[36]		[36]				
H. dujardini						15 [37]	0.8 [38]		
R. varieornatus						0.6 [18]	0.97 [39, 18]		

Supplementary Figure 1: Genomic features studied in asexual genomes. The figure mirrors the data from Figure 1, but with references to specific studies added (numbers correspond to the references cited in the main 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 by Handoo et al. [117]. Our analyses indicate that *M. floridensis* is triploid rather than diploid (**Supplementary Figure 2**), and the heterozygosity detected in our and previous studies [117] 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* [118]). 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. [117] 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 images of karyotypes or egg cells at the basis of their conclusions. 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”.

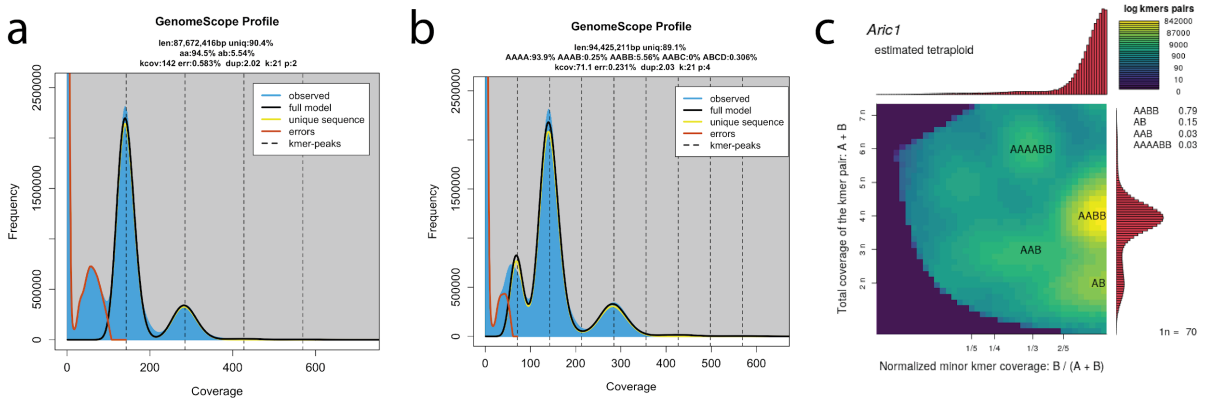


Supplementary Figure 2: Genomic evidence of triploidy in *M. floridensis*. a | the smudgeplot shows dominance of a triploid (AAB) genome structure. The smudges corresponding to higher ploïdies are likely originating from paralogs. The diploid kmer pairs (AB) represent situations where the third allele 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 Haplotype structure of *Adineta ricciae*

The diploid genome assembly (i.e., where haplotypes are largely separated and present in the assembly) of *Adineta ricciae* spans 201 Mbp and carries approximately 63,000 genes [19]. Analyses of assembled genome sections suggested a tetraploid genome structure AABB similar to other bdelloid rotifers [19,61]. However, our reanalyses of the *A. ricciae* sequencing reads with kmer spectra appear to be better consistent with an octoploid structure, rather than tetraploid. The interpretation of kmer spectra in rotifers is complicated by the fact that the A and B genomes are very highly diverged (in *A. ricciae*, the average divergence between A and B is 33.21%, while the divergence within AA and BB is 5.55%; **Figure 2**). Given this extreme divergence, A and B share practically no kmers and therefore the kmer spectrum is expected to miss the homozygous peak at 4n and therefore resemble the spectrum of a diploid species (with high heterozygosity). However, if *A. ricciae* was indeed characterized by a tetraploid AABB genome structure, the kmer spectrum should show a big haploid (n) peak at 124x coverage (as calculated from the genome size and total sequencing reads), generated from variation within A and B haplotypes. A second (2n) peak at ~248x would then be generated by homozygous regions within A and B. However, the kmer spectrum of *A. ricciae* does not follow these predictions (**Supplementary Figure 3a**). Instead, we observe three peaks: a haploid peak at 71x coverage (partially overlapping with variation generated by sequencing error), a diploid peak at 142x and a third, tetraploid peak at ~284x (**Supplementary Figure 3b**). We verified that the 1n peak indeed contains complementary kmers and therefore is not generated by sequencing errors or contamination (**Supplementary Figure 3c**). If it is the case that homoeologous kmers are entirely excluded from the kmer analyses, the resultant spectrum with three peaks (n=71x, 2n~142x and 4n~284x) is incompatible with the proposed degenerate tetraploid structure based on the current interpretation of the genome assembly [19]. Rather, these patterns are better explained by a 2x higher ploidy level, i.e., to octoploidy. A simple

doubling in chromosome number in *A. ricciae* is not supported by the observation of 12 chromosomes in this species, the same as for *A. vaga* [119], and thus any increase in ploidy level in *A. ricciae* would seem to be either complex or very recent. Alternatively, the kmer peak at ~284x might be caused by collapse across all four subgenomes, creating regions of the genome with 0% divergence across both homologous and homoeologous copies. However, this hypothesis is at odds with observations of read- and SNP-coverage from [19], that indicate the majority of the diploid assembly (~81%) is twofold covered, consistent with the idea of large-scale increases in ploidy level. Finally, it is not entirely precluded that the raw data itself, or the methods used to generate it, contains some stratification that might produce a false signal of increased ploidy. Further investigation of these alternative hypotheses will likely require additional data, such as long-read sequencing technology, which is beyond the scope of the present study. However, the likely differences in ploidy levels between *A. vaga* and *A. ricciae* preclude a clear interpretation of the strikingly different allele divergences between these two species.



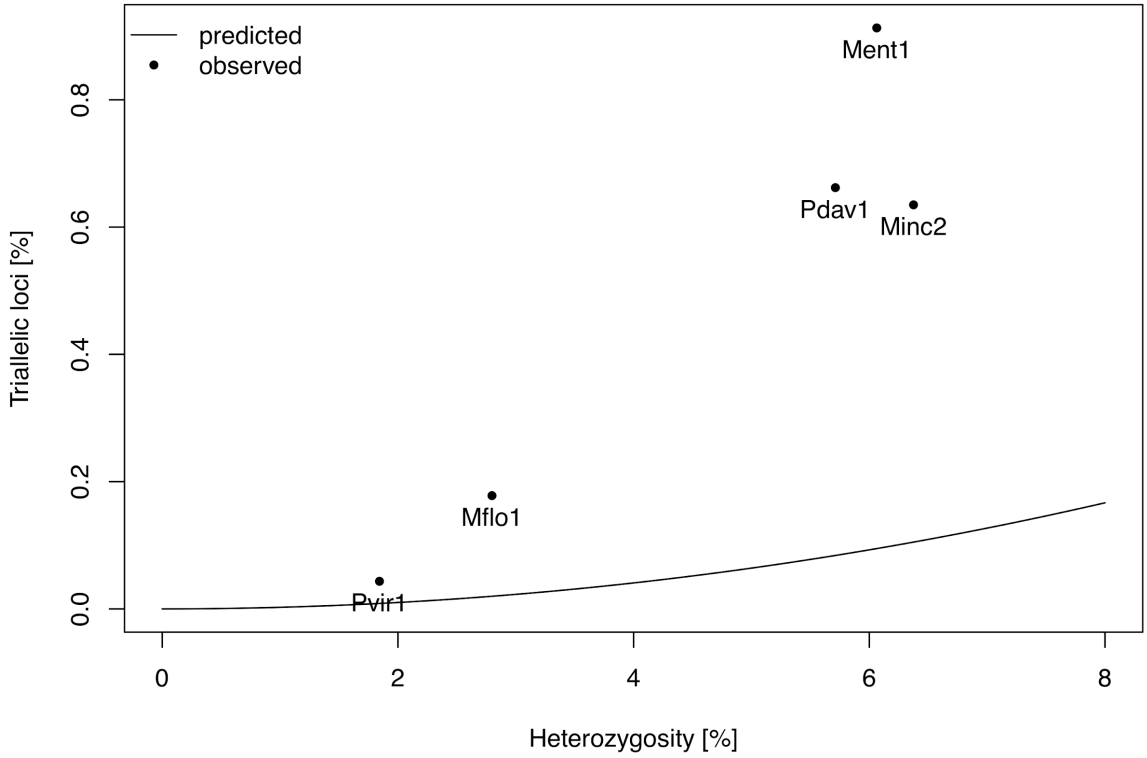
Supplementary Figure 3: Genome profiling of *A. ricciae*. a | Genome model used in our study. **b** | The best fit genome model supporting degenerate tetraploidy, which, assuming homoeologs have no shared kmers in the rotifers, corresponds to degenerate octoploidy (for a more detailed interpretation see Supplementary Material S2) **c** |

smudgeplot of *A. ricciae* indicating a 70x haploid coverage, and supporting the genome model shown on panel b.

S3 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. Assuming heterozygous alleles are randomly distributed across the genome we can generate a naive expectation of the fraction of triallelic loci in triploid species (in the absence of fitness effects linked to heterozygosity). This expectation is dependent on two variables: 1) how symmetric the pairwise divergences between the three genomic copies are, and 2) the total heterozygosity levels. In the main text (Figure 3) we report variation among triploid species in the frequency of bi- and triallelic loci. To verify that this variation is not solely generated by different total heterozygosity levels, we compared the observed proportion of triallelic loci among species while taking the bias generated by total heterozygosity levels into account. Consider a reference genome copy and two genome copies with divergences to the reference d_1 and d_2. The total heterozygosity (h), as defined in **Box 3**, of the genome is $h = d_1 + d_2 - d_1d_2$ and can be further decomposed into biallelic heterozygosity ($d_1 + d_2 - 2d_1d_2$), and the triallelic heterozygosity (d_1d_2) as the overlap between the divergences d_1 and d_2. Note that the overlap is the highest in a genome with equidistant divergence of the genomic copies ($d_1 = d_2$). Given a heterozygosity *h*, the expected triallelic heterozygosity can be expressed as $d_{tri} = (1 - \mathrm{sqrt}(1 - h))^2$.

This expectation does not correspond to the biological reality as genomes contain many regions with elevated or reduced heterozygosity but it allows us to compare genomes while correcting for the bias generated by different total heterozygosity levels. The frequencies of triallelic loci remain highly variable among the five triploid species even after correcting for this bias (**Supplementary Figure 4**), meaning the conclusions in the main text are supported even when we correct for total heterozygosity levels.

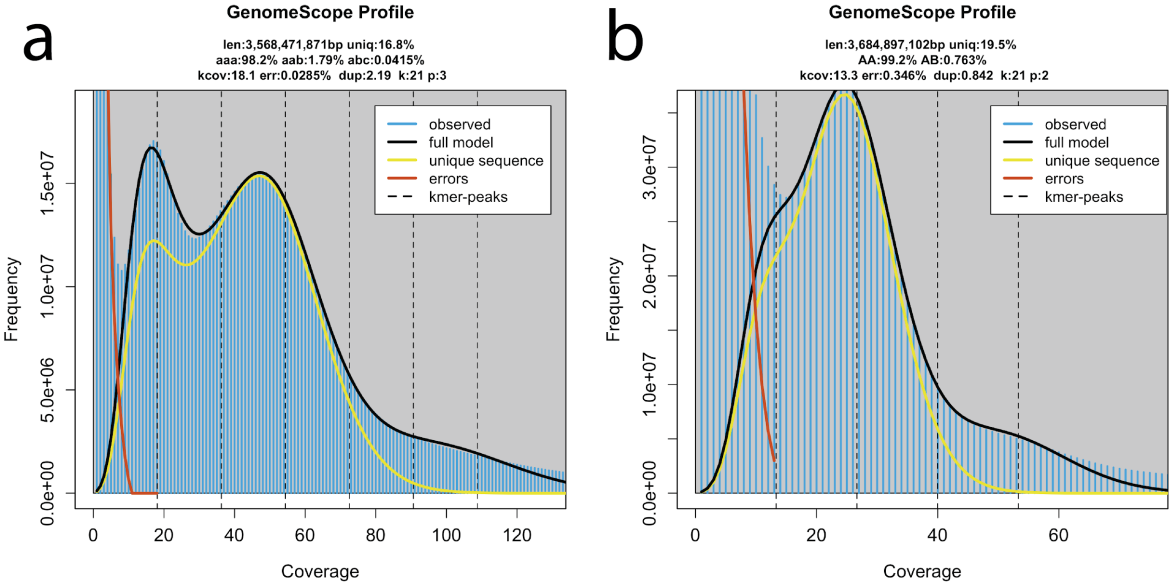


Supplementary Figure 4: Fraction of triallelic loci according to the total heterozygosity. We observe two distinct groups of triploid species. The crayfish (Pvir1) and *M. floridensis* (Mflo1) have a much smaller proportion of triallelic loci than the three other species, even when values are adjusted for a bias generated by total heterozygosity. The expectation was calculated as the overlap of heterozygosity in a genome with equidistant divergence (See Supplementary Materials S3)

S4 Suggestive hybrid origin of the marbled crayfish

Previously it has been suggested that the triploid asexual crayfish *Procambarus virginalis* is an autopolyploid linage derived from diploid sexual *P. fallax* [120]. The main arguments for autopolyploidy (instead of allopolyploidy) are that *P. virginalis* and *P. fallax* are morphologically very similar and that *P. virginalis* does not carry any morphological trait of any other closely related crayfish. Our analysis revealed two nearly identical genome copies (**Figure 3**) supporting endoduplication (i.e., autopolyploidy) as a source of triploidy. However, it also revealed the presence of a highly diverged genome copy, suggesting hybridization between at least highly diverged strains or populations, if not species.

Specifically, the heterozygosity estimate for the asexual triploid *P. virginalis* is ~1.8% (**Supplementary Figure 5a**). 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 5b**). 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*.

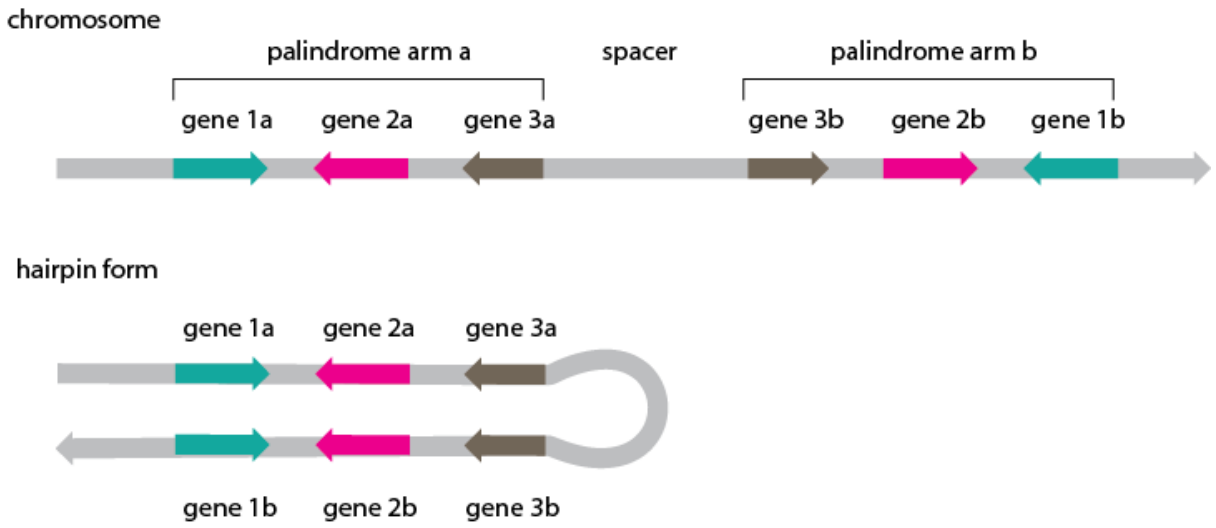


Supplementary Figure 5: Genome profiling in crayfishes. a | A triploid genome model in asexual *P. virginalis* estimates heterozygosity to 1.79%. **b |** A diploid genome model in its sexual sister species *P. fallax* estimates a similar genome size, but substantially lower heterozygosity (0.76%). However, the quality of fit is less conclusive as the error peak (red) and haploid peak (leftmost black) largely overlap.

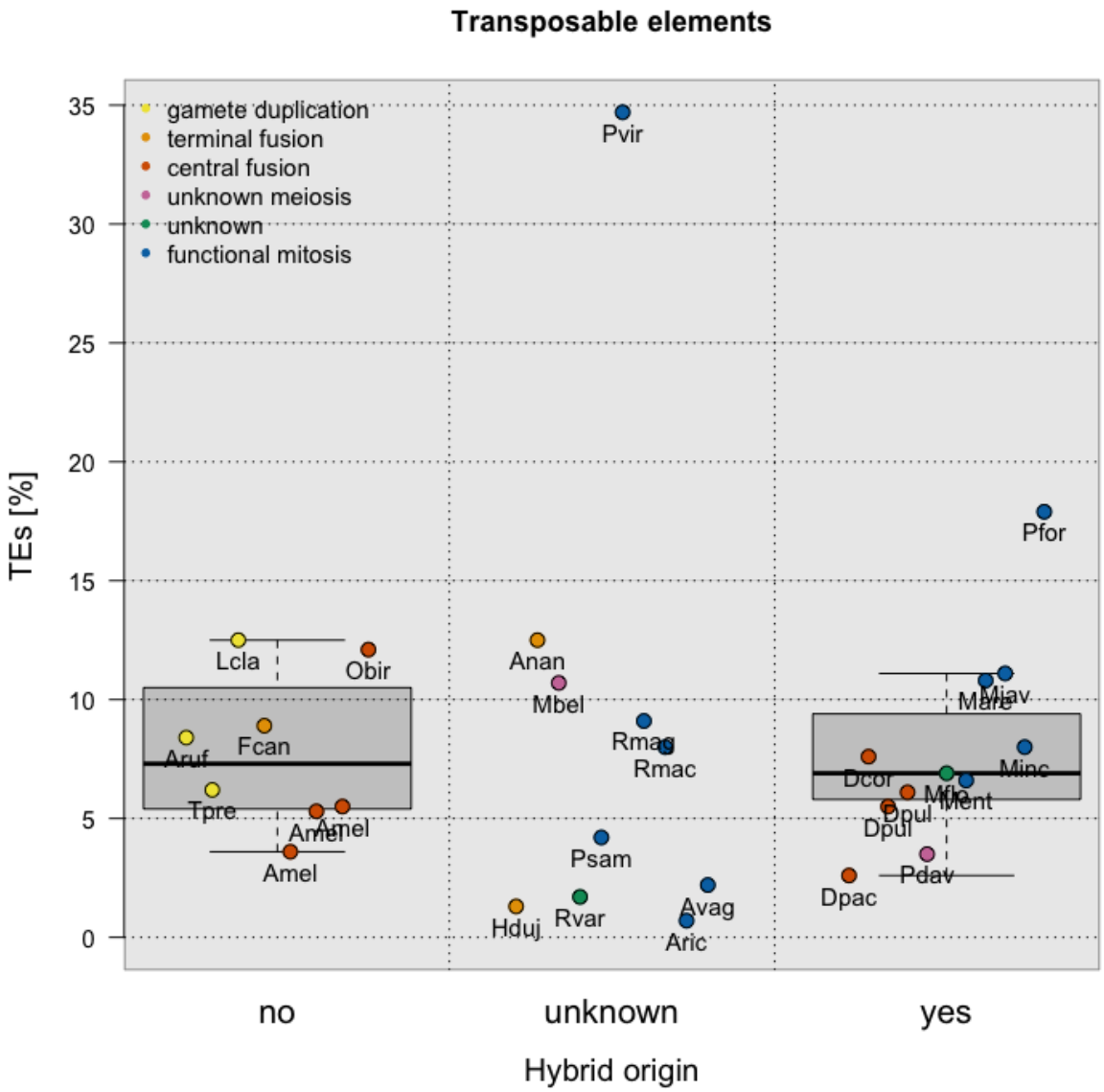
S5 Palindrome detection

Palindromes are formed of two homologous reverse complementary sequences on the same chromosome (**Supplementary Figure 6**). Palindromes can facilitate gene conversion and therefore help to escape mutational meltdown via Muller's ratchet [64,65]. To test if they play such a role in asexual organisms we identified palindromes using colinearity analysis imlplemented in the program MCScanX [114]. The default parameters of the software (used in the genome studies of asexual species, personal communication of the authors of [18,26]) 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 6**).

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 6: 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 7: Transposable elements with respect to reproduction mode and hybrid origin. Neither hybrid origin (*p*-value = 1) nor cellular mechanism of asexuality (*p*-value = 0.84) are strong drivers of the TE content in asexual animals.

S6 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 (i.e., highly conservative) 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 higher for some other taxa, see **Supplementary Table S4**), highlighting the sensitivity of such analyses on the sequence databases used. The proportion of HGT_C observed in bdelloid rotifers also dropped, relative to comparisons against UniRef (3.64 - 5.11%), but remain high relative to other taxa. The impact of the specific database used for HGT detection may be driven by potential taxonomic misclassifications and/or contamination in one or both public databases, or biases in the representative taxa contained within particular databases (e.g., UniProtKB/Swiss-Prot is biased towards a relatively small number of model species). A full investigation of the true composition of public sequence repositories is a major piece of work that is beyond the scope of the current work.

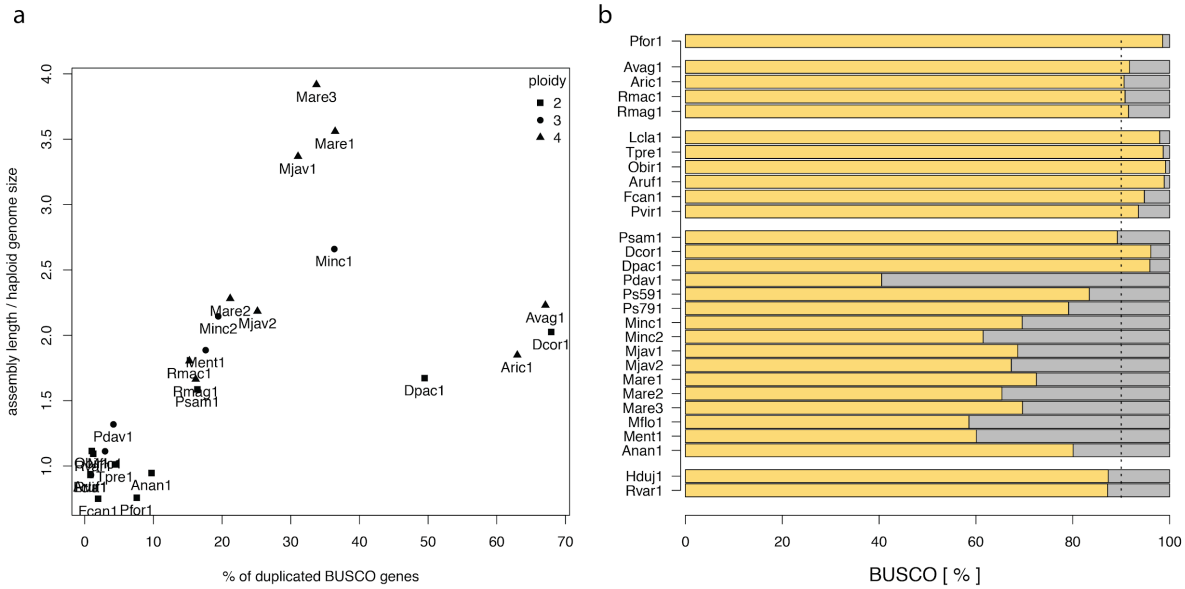
Overall our analysis showed that high levels of HGT are not a general feature linked to asexuality but a clade specific trait of bdelloid rotifers and perhaps of hexapods. However, it is important to note that our analysis does not quantify absolute levels of HGT in any of the taxa; instead, we show the dependency of such estimates on the chosen methodology.

S7 Variation in estimated TE loads

We estimated the TE loads in each genome via assembly of overrepresented (i.e., repetitive) genomic sequences and subsequent annotation via homology searches in general databases (see methods). This can result in an underestimation of TE loads in species with many TE types with very low copy numbers (as is the case in *A. ricciae* and *A. vaga* [18–20]) and in phylogenetically isolated lineages such as rotifers and tardigrades which may comprise divergent TEs not represented in general databases. However, this is unlikely to be the sole reason behind the low TE content of asexuals reported in our study since the methods we used allowed us to identify similar or higher TE loads in all species without manually curated TE libraries (**Figures 1 and 4**). Manual curation (available for *D. pulex* and *A. vaga* among the species in **Figure 1**) generally increases TE load estimations. For example, manual curation of TEs in the *Daphnia* genome increased estimated TE loads from approximately 9.6% to 17-27% [121]. In *A. vaga*, the recent discovery of a new TE family during manual curation increased the latest TE load estimate to nearly 4% [20].

S8 Conserved gene content

We aimed to provide insights into gene duplications and losses by quantifying conserved single copy orthologs (BUSCO genes) [122]. 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 generating 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 8a**).



Supplementary Figure 8: 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.

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.

species	common name	NCBI accesion	reproduction mode	evidence	hybrid origin	age of asexuality [y]	references
Poecilia formosa	amazon molly	SAMN01797685	sperm-dependent apomixis	cytology, genetics	yes	100 k	[1]
Adineta vaga	bdelloid rotifer	SAMEA2043852	apomixis	cytology		46 M	[2,3]
Adineta ricciae	bdelloid rotifer	SAMEA104393659	apomixis	cytology		46 M	[3,4]
Rotaria macrura	bdelloid rotifer	SAMEA104393678	apomixis	no direct evidence[1]		46 M	
Rotaria magnacalcarata	bdelloid rotifer	SAMEA104393684	apomixis	no direct evidence[1]		46 M	
Leptopilina clavipes	parasitoid wasp	SAMN02047179	gamete duplication[2]	cytology	no	6-43 k	[5]
Trichogramma pretiosum	trichogramma wasp	SAMN02439301	gamete duplication[1]	cytology, genetic markers	no	"few"	[6,7]
Ooceraea biroi[3]	raider ant	SAMN02428046	central fusion automixis	cytology, RAD seq	no		[8]
Apis mellifera capensis	cape honey bee	SAMN10245904 SAMN10245905 SAMN10245906	central fusion automixis	cytology	no	20	[9]
Aptinothrips rufus	thrip		gamete duplication[4]		no	150-200 k	[10]
Folsomia candida	springtail	SAMN04196550	terminal fusion automixis[3]	cytology	no		[11]
Daphnia pulex	water flea	SAMN03964753 SAMN03964750	central fusion automixis equivalent	No separation at meiosis I; abortive meiosis	yes	1-170 k	[12]
Procambarus virginalis	marbled crayfish	SAMN07142640	apomixis	microsat study, histological evidence		less than 30	[13–15]
Plectus sambesii	nemotode	SAMN07227113	endoduplication	2 meiotic divisions, 2 polar bodies, but no fusions were observed			[16]
Mesorhabditis belari	nemotode	SAMEA5150020	unknown automixis	cytology; 2 meiotic divisions observed			[17]

[1] Meiosis was not observed in any of the bdelloid rotifers including *Philodina roseola*, a member of *Philodinidae*, the same family as *Rotaria*.
[2] *Wolbachia* induced
[3] formerly *Cerapachys biroi*
[4] suggested that it's endosymbiont induced

Diploscapter coronatus	nemotode	SAMD00025087	automixis central fusion	Only 1 meiotic division, given heterozygosity, must be separation of chromatids (would probably mean meiosis is inverted)	yes		[16, 18]
Diploscapter pachys	nematode	SAMN03456257	central fusion automixis equivalent	meiosis I skipped, no recombination, only sister chromatid separation - a model	yes	18 M[5]	[19]
Panagrolaimus davidi	nemotode	SAMN02741088	unknown automixis	cytology; polar body produced	yes	1.3-8.5 M	[20]
Acrobeloides nanus	nemotode	SAMN06041019	terminal fusion equivalent				[16]
Meloidogyne incognita	root-knot nematode	SAMEA104032784 SAMN05712521	apomixis		yes	"recently"	[21–23]
Meloidogyne javanica	root-knot nematode	SAMEA3298191 SAMN05712519	apomixis		yes	"recently"	[23]
Meloidogyne arenaria	root-knot nematode	SAMEA3298190 SAMN05712513 SAMN08721831	apomixis		yes	"recently"	[23]
Meloidogyne floridensis	peach root-knot nematode	SAMN05712529	unknown automixis	cytology; mismatch in ploidy (see S1)	yes	"recently"	[23, 24]
Meloidogyne enterolobii	root-knot nematode	SAMN05712528	apomixis		yes	"recently"	[23]
Hypsibius dujardini	tardigrade; water bear	SAMEA3679301	automixis terminal fusion equivalent	meiosis II suppressed			[25]
Ramazzottius varieornatus	tardigrade; water bear; Kumamushi	SAMD00054187		no males have been found			[6]

[5] Assuming non-hybrid origin suggested by Hiraki at al. 2017.
[6] personal communication with Mark Blaxter

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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*.

Supplementary Table 4: A complete overview of horizontal gene transfer analysis.
https://github.com/KamilSJaron/genomic-features-of-asexual-animals/blob/master/tables/HGT_table.tsv