bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Genomic features of asexual animals

Kamil S. Jaron[1, 2], Jens Bast[1], T. Rhyker Ranallo-Benavidez[3], 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 Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA

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 have been linked to asexuality, including high heterozygosity, a high abundance of horizontally acquired genes, a low transposable element load, and the presence of palindromes. However, it is unclear whether these features are lineage-specific or general consequences of asexuality. We reanalyzed published genomes of 24 asexual animals and found that not a single genome feature is systematically replicated across a majority of these species, suggesting that there is no genomic feature characteristic of asexuality. We found that only asexuals of hybrid origin were characterized by high heterozygosity levels. Asexuals that were not of hybrid origin appeared to be largely homozygous, independently of the cellular mechanism underlying asexuality. Overall, despite the importance of recombination rate variation for understanding the evolution of sexual animal genomes, the genome-wide absence of recombination does not appear to have the dramatic effects which are expected from classical theoretical models. The reasons for this are probably a combination of lineage-specific patterns, impact of the origin of asexuality, and a survivor bias of asexual lineages.

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Introduction

Sex: What is it good for? The reasons for 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 that use asexual reproduction as their sole form of replication typically occur at the tips of phylogenies and only few of them have succeeded like their sexually reproducing counterparts [3]. In other words, most asexual lineages may eventually be destined for extinction. These incipient evolutionary failures are however invaluable because by understanding the evolutionary fate of asexual species, something may be learned about the adaptive value of sex.

An accumulating number of studies have sequenced the genomes of asexually reproducing animals, often with the aim of identifying features that would distinguish them from sexual species (**Figure 1**). In asexual animals, females produce daughters from unfertilized eggs via so-called thelytokous parthenogenesis (hereafter asexuality) [4]. Asexuality is predicted to have many consequences for genome evolution, since gamete production via meiosis and the restoration of somatic ploidy levels via fertilization no longer take place. Predicted consequences include for example the accumulation of deleterious mutations [5–7], as well as changes in heterozygosity levels [8,9] and transposable element (TE) dynamics [10]. Some predictions have been tested without genomic data, using a handful of housekeeping genes. However, conclusions based on such small and non-random subsets of genomes can lead to erroneous conclusions [11]. With the advent of high-throughput sequencing it is possible to evaluate classical predictions of asexuality at the genome scale, and furthermore to test new predictions, such as the accumulation of palindromes (see below), which could not be studied with single gene approaches. In the present study, we compare the published genomes of 24 asexual animal species (**Figure 1**) to assess whether we can identify any key features characteristic of asexual animals. The 24 species comprise four species of bdelloid rotifers, a group that likely persisted and diversified in the absence of canonical sex for over 40 million years [12]. Bdelloids have thus far overcome the predicted dead-end fate of asexuality, which raises the question of what mechanisms protect them from extinction, and whether these mechanisms are visible in specific characteristics of their genomes.

Because the predicted consequences of asexuality are strongly affected by how asexuality evolved from the sexual ancestor (**Box 1**) as well as by the cellular mechanisms underlying asexuality (**Box 2**), we include biological differences among asexual species in our comparisons. For example, some asexual species have evolved via hybridization (**Box 1**), which generates high heterozygosity and can result in increased activity of transposable

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

elements [13–15]. In such instances, it can be difficult to disentangle consequences of hybridization from consequences 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 (**Box 2**). Finally, because genome studies differed in their focus and in the methods used, we reanalyzed 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 not a random nor representative sample of asexual animals, and that not all of these genomes reflect evolutionarily independent events.

We uncovered a number of unusual features in the genomes of asexual animals, including extreme loads of transposable elements and highly 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. We found that a hybrid origin of asexuality was the most important factor affecting heterozygosity, with potential effects of asexuality being masked by effects of hybrid ancestry. Unexpectedly, asexuals that are not of hybrid origin are largely homozygous, independently of the cellular mechanism underlying asexuality.

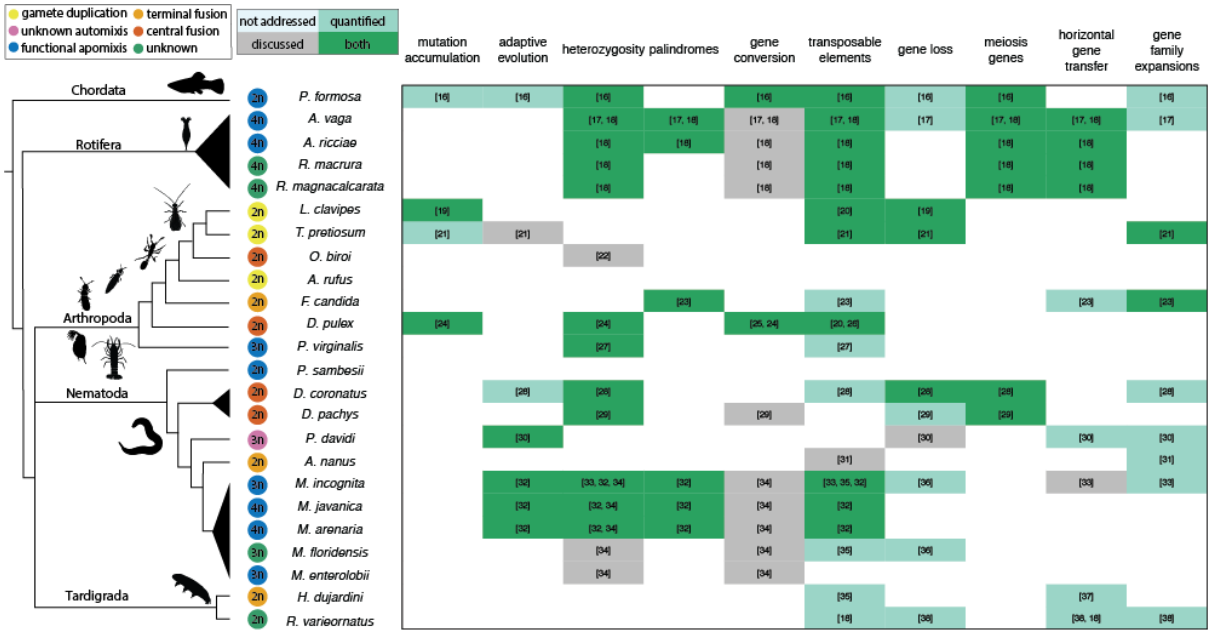


Figure 1: **Genome features studied in asexual animal species.** The phylogeny displays the taxonomic relation of the 24 sequenced asexual animal species considered here. The color of the circle indicates the cellular mechanism of asexuality and the number inside the

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

circle the ploidy of the species (see Supplemental Table 1 for details). Note that *M. floridensis* is considered 2n in the published genome studies but our analyses clearly show that this species is triploid (Supplementary Materials). The 24 species correspond to at least 16 independent transitions from sexual to asexual reproduction; species that might derive from the same original transition are grouped in triangles. Each original genome article explored a given set of genome features: the green cells (both light and dark) represent cases where the genomic feature was quantified; the dark cells (grey, dark green) represent studies where the genomic features were discussed with respect to asexuality. Heterozygosity, palindromes and transposable elements were reanalysed in this study, the discussion of the remaining features is based on the analyses reported in the individual genome studies [16–38].

Box 1: Transitions to asexuality

Meiotic sex evolved once ~1.5 billion years ago, and since then remained the predominant mode of reproduction in eukaryotes [3,39]. Current asexual animals therefore derive from a sexual ancestor, but how transitions from sexual to asexual reproduction occur can vary. While the molecular changes underlying different types of transitions are unknown, the expected genomic consequences of asexuality vary extensively among them [14].

Hybrid origin. Hybridization between sexual species can generate hybrid females that reproduce asexually [14,40]. Asexuality caused by hybridization generates high levels of heterozygosity, corresponding to the divergence between the parental sexual species prior to hybridization. Hybridization can also result in a burst of transposable element activity [13].

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

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

In sexual species offspring is 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 cellular mechanism used is predicted to affect genome evolution and especially heterozygosity levels. For details see [4,42].

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

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

Endoduplication. A duplication of the entire chromosome set occurs before normal meiosis, during which ploidy is reduced again. If recombination occurs between identical chromosome copies rather than between chromosome homologs, endoduplication produces offspring that are clones of their mother.

Inverted meiosis with terminal fusion (gonoid thelytoky). During the first meiotic division, sister chromatids separate instead of homologous chromosomes. The homologues are separated in the second meiotic division. In the absence of recombination, inverted meiosis with terminal fusion generates offspring that are clones of their mother (and though mechanistically different, is conceptually equivalent to central fusion without recombination). Holocentric chromosomes seem to be a prerequisite for this type of mechanism [43,44].

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.

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

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Results

Overview of species and genomes studied

We reanalyzed the published genomes of 24 asexual animal species with the aim of identifying general genomic signatures of asexualilty. The 24 species correspond to at least 16 independent transitions to asexuality and cover a broad taxonomic range, including chordates, rotifers, arthropods, nematodes and tardigrades. In addition to covering this taxonomic range, the 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 were studied in 20 of the 24 species. Eight of them use mitotic asexuality, while the 12 remaining species use different types of meiotic asexuality (**Figure 1**). All but one of the eight species with mitotic asexuality are polyploid, the amazon molly *P. formosa* 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 sp.* is characterized by both meiotic asexuality and triploidy [30] (see **Supplementary Table 1** for details).

Information on how asexuality evolved is available for 15 of the 24 sequenced species (Figure 1). A hybrid origin has been suggested for ten of these. Endosymbionts are the most likely cause of asexuality in four species (the springtail, both wasps and the thrips), and spontaneous mutation in one (the ant). Across the 24 species, 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 has thus far not been suggested, but is supported by our results (see below). It is important to note however that there are many polyploid asexual animals that 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 increases in old asexual

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

lineages. However, estimating the age of asexual lineages is difficult and always associated with large uncertainties [46,47]. We therefore did not include quantitative comparisons among asexuals with respect to their age. However, because our set of species comprises asexuals believed to be 'ancient' (i.e., several million years old, see **Supplementary Table 1**), we discuss, where appropriate, potential age effects in a qualitative manner.

Mutation accumulation and positive selection

One of the classical predictions linked to asexuality is that it reduces the efficacy of selection [5–7,48–50]. This reduction occurs because linkage among loci in asexual species prevents selection from acting individually on each locus. This can allow for 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.

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

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

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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

Expected heterozygosity levels in asexual organisms are influenced by 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 should increase or decrease over time) (**Box 2**), and (3) for how long a species has been reproducing asexually (because effects of asexuality accumulate over time).

As expected, all of the asexual species with a known hybrid origin display high heterozygosity levels (1.73% - 8.5%, **Figure 2**), while the species with an intraspecific origin of asexuality show low heterozygosity levels (0.03% - 0.53%, **Figure 2**). However, it is important to note that hybrid origin is correlated with polyploidy in our dataset, and that heterozygosity does not have a clear definition in polyploids **(Box 3)**. Our measures of heterozygosity are based on the proportion of sites with more than one allele present among all copies, where the total number of copies includes all homologous genome regions **(Box 3)**.

The heterozygosity levels present at the inception of asexuality should decay over time for most forms of meiotic asexuality [42,55]. Under mitotic asexuality, heterozygosity is expected to increase over time as haplotypes can accumulate mutations independently of each other (generating the so-called 'Meselson effect')[8]. However, gene conversion can strongly reduce haplotype divergence and, if high enough, can even result in a net loss of heterozygosity over time, even under mitotic asexuality [8,17]. In spite of the prediction that the cellular mechanism of asexuality should affect heterozygosity, the cellular mechanism of asexuality appears to have little or no 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. Nevertheless, it is interesting to note that species with

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

different forms of meiotic asexuality (including gamete duplication and central fusion) feature 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 picked up with our methods.

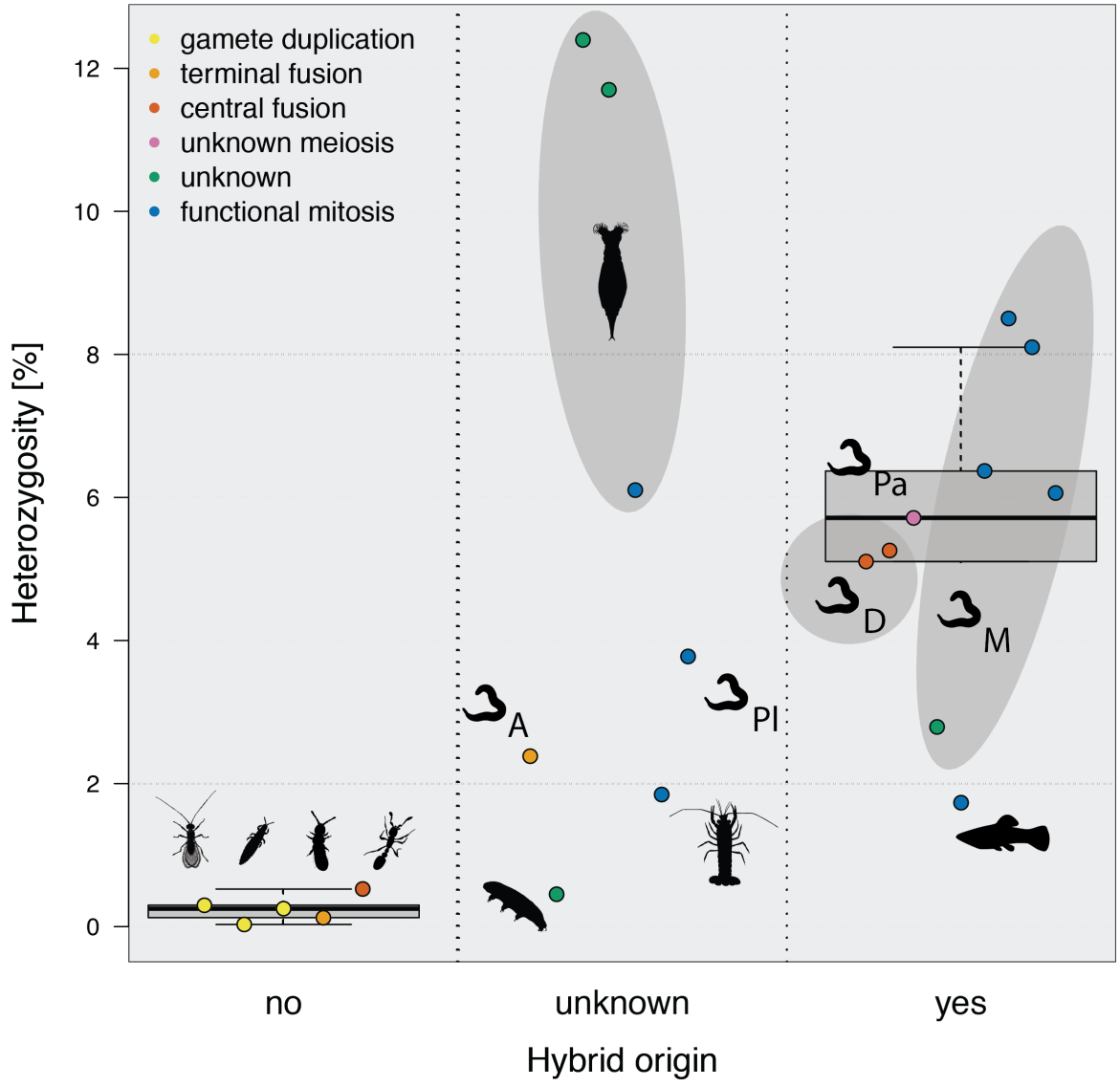


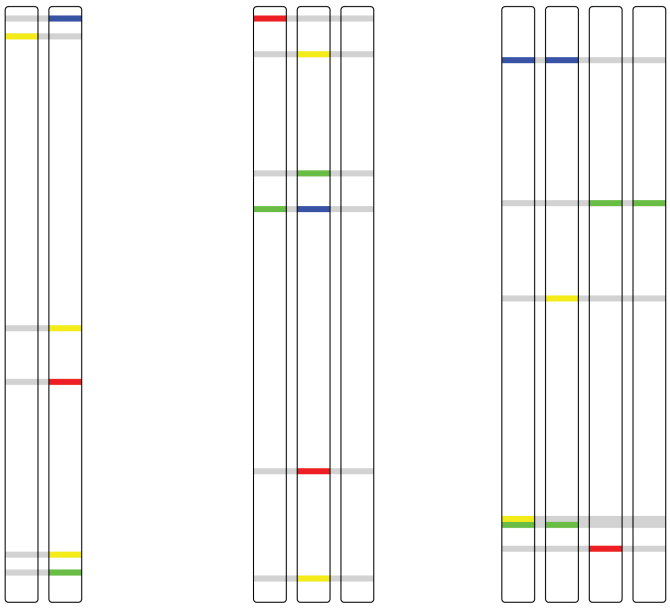
Figure 2: **Hybrid origin is the main driver of high heterozygosity in asexual species**. Heterozygosity estimates with respect to hybrid origin (x axis) and cellular mechanism of asexuality (color code). Species with a possible shared origin of asexuality are grouped in gray ellipses. Nematode genus abbreviations: Pl: *Plectus*, D: *Diploscapter* Pa: *Panagolaimus*, A: *Acrobeloides*, M: *Meloidogyne*. We were unable to generate heterozygosity estimates for three of the 24 asexual species for different reasons: in the

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

tardigrade *H. dujardini* because of extensive contamination in the sequencing reads, in the water flea *Daphnia pulex* samples because of too low coverage, and in the rotifer *A. vaga* because of divergence levels that exceed the range quantifiable with the applied methods (see **Methods** and **Figure 3**). Heterozygosity is significantly higher in the eight asexuals of confirmed hybrid origin relative to the five that are not (Mann–Whitney U test; p-value = 0.0009).

Box 3: Quantification of heterozygosity for different ploidy levels

The classical definition of heterozygosity describes heterozygosity as a measure of allelic divergence, where alleles are defined through chromosome pairing [56]. This definition poses a problem for genomes where chromosome pairing is not known (e.g., in polyploid genomes), as well as for genomes of mitotic asexuals where chromosome pairing may not occur at all. In these cases, concepts such as divergence between alleles (i.e., heterozygosity) *vs.* divergence of paralogs, that are clearly distinct in diploids, become blurred. We therefore quantify heterozygosity as the proportion of nucleotides that differ in at least one of the homologous chromosomes. Three examples of genomes with similar heterozygosity but different ploidy levels are shown on the scheme for illustration. Grey bars highlight specific loci, coloured bars represent alternative alleles at a given locus.



bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Heterozygosity structure in polyploids

Heterozygosity in polyploids is estimated via the proportion of sites that differ in at least one of the homologous regions (see **Box 3**). This means that 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. We therefore decomposed genome-wide heterozygosity for each polyploid genome into portions with different divergence structures (**Figure 3**).

In polyploid species of hybrid origin (sexual or asexual), heterozygosity is generally driven by divergence between haplotypes originating from different species (hereafter homoeologs, following the terminology of Glover et al [57]). In our dataset, the polyploid species with confirmed hybrid origins are nematodes in the genera *Meloidogyne* (five species) and *Panagrolaimus* (one). As expected, heterozygosity in these species is largely dominated by divergence between homoeologs. In the triploid species, divergence is between a single homoeolog and two similar homologs (yellow portions in **Figure 3**), consistent with previous findings [30,34]. In the tetraploid species, genome-wide heterozygosity is generated by a combination of genome portions comprised of one homoeolog and three similar homologs and of other portions comprised of pairs of homoeologs (pink portions in **Figure 3**).

Given that in asexual polyploids of hybrid origin we expect and observe highly heterogeneous divergences among haplotypes, while polyploidy of intra-specific origin is predicted to generate homogeneous divergences, haplotype divergences can be used to infer the origin of asexuality in polyploid species. Notably, the highly asymmetric divergence levels between haplotypes in the four bdelloid rotifers (**Figure 3**) are best explained by a hybrid origin of bdelloids. When tetraploidy was first discovered in bdelloids, it was proposed that tetraploidy stemmed from either a whole genome duplication or a hybridization event in their ancestor [58]. However, studies of bdelloid rotifers traditionally refer to the divergent haplotypes as “ohnologs” (e.g., [17,18]), which, following the unified vocabulary of Glover et al [57] would imply that the diverged haplotypes are products of a whole genome duplication. However, given their likely hybrid origin, referring to them as homoeologs appears more appropriate.

Whether the crayfish is also of hybrid origin remains as an open question. Its genome features two nearly identical haplotypes and one that is substantially divergent (1.8%, **Figure 3**), which is suggestive of a hybrid origin. However, the divergence of the latter is within the

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

range of heterozygosity commonly observed in sexual species, and therefore we cannot clearly distinguish between an intra-specific or a hybrid origin.

Our analyses also reveal that the divergence of homologs varies extensively among bdelloid rotifer genera. Divergence is very low in *Rotaria* (0% in *R. magnacalcarata* and 0.25% *R. macrura*) and low in *A. ricciae* (0.5%) but relatively high in *A. vaga* (2.4%). The mechanisms causing these differences remain unknown. In *A. vaga* it has been suggested that gene conversion reduces divergence between homologs in some genome regions [17]. It is possible that rates of gene conversion are higher in *Rotaria* than *Adineta*, for unknown reasons. Independently of the mechanisms causing the differences between bdelloid genera and species, it is important to note that with such low levels of divergence between homologs, there can be no genome-wide 'Meselson effect' in bdelloid rotifers (see also [17]). It remains possible that the subset of genomic regions with divergence between homologs in *Adineta* feature allele phylogenies as expected under the 'Meselson effect'. This is the case in the asexual unicellular eukaryote *Trypanosoma brucei gambiense*: some genome regions feature high heterozygosity and allele phylogenies as expected under the 'Meselson effect', while others are largely homozygous [59]. Again, it remains unknown why there is such extensive heterogeneity in divergence across the genome in this species. A possible explanation is that the heterozygous genome regions are the consequence of ancient introgression, and that gene conversion rates are low in such regions with very high heterozygosity (see Discussion).

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

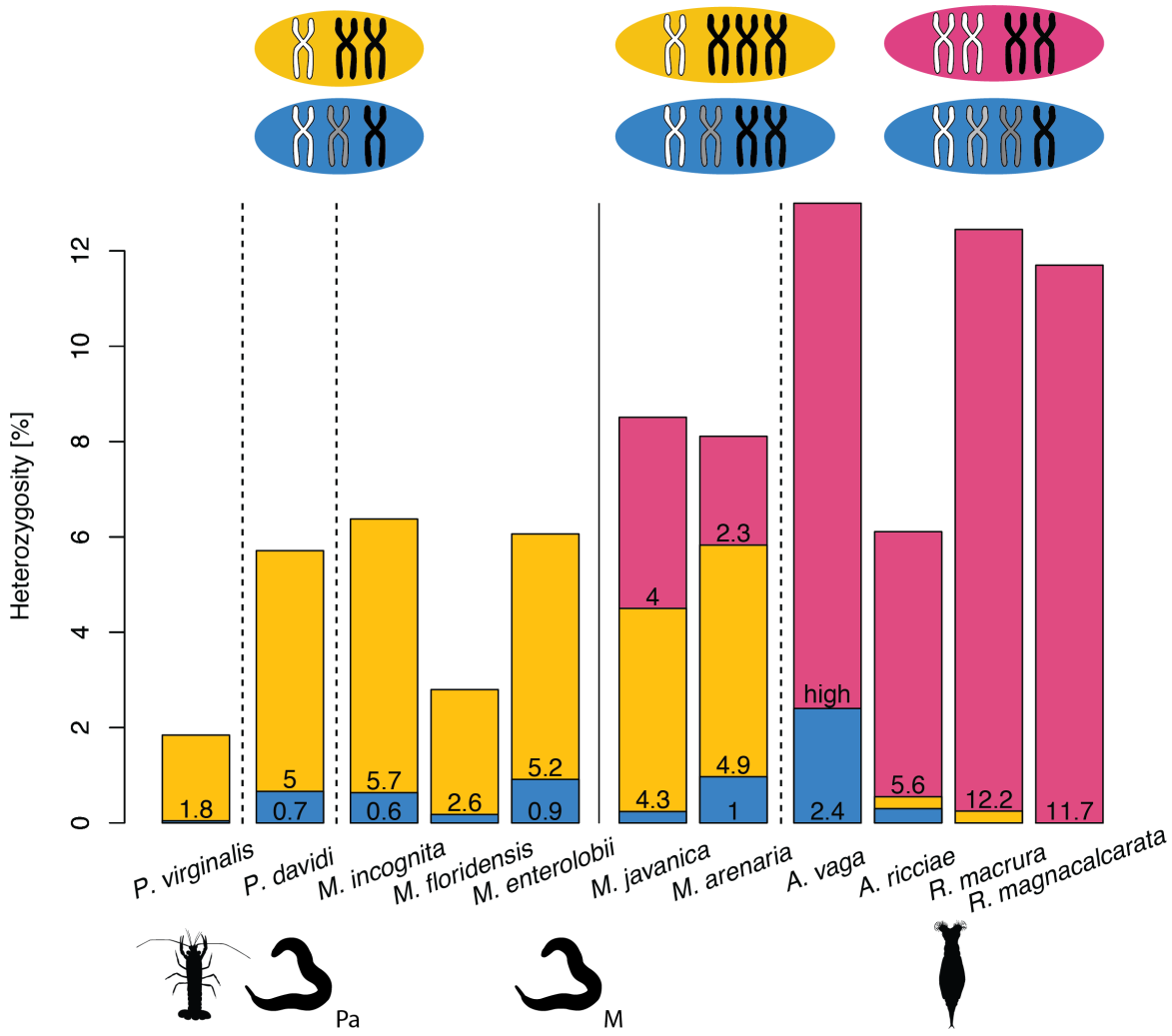


Figure 3: **Heterozygosity structure in polyploids.** Biallelic loci are indicated in yellow or pink:, yellow when the alternative allele is carried by a single haplotype (AAB or AAAB), and pink when both alleles are represented twice (AABB). Loci with more than two alleles are indicated in blue. Note that homoeolog divergence in the rotifer *Adineta vaga* is so extensive that it is impossible to estimate the exact divergence level 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 2**). Palindrome-mediated gene conversion was shown to play a major role in limiting the accumulation of deleterious mutations for non-recombining human and chimpanzee Y chromosomes [60–62]. Indeed, approximately one third of coding genes on these Y chromosomes occur in palindromes, and the highly concerted evolution of palindromic regions indicates that the rates of gene conversion are at least two orders of magnitude higher in the palindromes than between

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

373 homologous chromosomes. The reports of palindromes in the genomes of the bdelloid rotifer
374 *Adineta vaga* [17] and the springtail *Folsomia candida* [23] led to the hypothesis that palindromes
375 could play a similar role in asexual organisms – reducing deleterious mutation accumulation
376 in the absence of recombination. However, the potential benefit of palindrome-mediated
377 gene conversion depends on the portion of genes in palindromic regions [61]. In addition to
378 identifying palindromes, it is therefore important to also quantify the number of genes
379 affected by palindrome-mediated gene conversion.
380
381 Methods for palindrome identification depend on genome assemblies (contrary to the other
382 genome features we re-analysed in our study). Palindromes are less likely to be detected in
383 highly fragmented assemblies and artificial palindromes can be generated by erroneous
384 scaffolding (see also [18]). Our analyses and interpretations assume that there are no
385 systematic scaffolding errors in the published assemblies. Palindrome identification methods
386 rely on genome annotations, which are available for 22 of the 24 asexual species (all except
387 *D. pulex* and *A. rufus*). We screened these 22 genomes for the presence of palindromic
388 arrangements (See **Methods** and **Supplementary Text S2** for details). We identified 19
389 palindromes in *A. vaga*, 16 in *F. candida,* and up to four palindromes in seven additional
390 genomes (Table 1). Not a single palindrome was detected in the remaining 13 species. The
391 frequency of palindromes had no phylogenetic signal; for example, although we found 19
392 palindromes in *A. vaga*, we found no palindromes in the three other bdelloid rotifers (in
393 agreement with [18]). There is also no indication for major rearrangements being present
394 solely in very old asexuals; among the very old asexuals, the non-*A. vaga* rotifers along with
395 the *Diploscapter* nematodes have either no or only a single palindrome.
396
397 *Adineta vaga* and *F. candida* are the only two species with more than 100 genes potentially
398 affected by palindrome-mediated gene conversion, but even for these two species, the
399 overall fraction of genes in palindromes is very small (1.23% and 0.53% respectively). The
400 fraction of genes in the other seven species ranges between 0.01% and 0.16%, suggesting
401 that palindromes do not play a major role in the genome evolution of any of the asexual
402 lineages analyzed. Our findings substantiate the conclusion of a previous study [18] that major
403 genomic rearrangements and the breaking of gene syntenies do not occur at high rates in
404 asexual organisms. They appear to occur at rates similar to those known in recombining
405 genome portions of sexual species [63,64].
406
407 **Table 1: Palindromes in asexual genomes.** Only species with at least one palindrome
408 detected are listed in the table. Rows in bold highlight species with more than 100 genes
409 detected in palindromes.

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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**
D. pachys	1	2	0.01
M. incognita	1	26	0.06
M. arenaria	3	38	0.04
H. dujardini	1	8	0.04
R. varieornatus	4	22	0.16

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

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

Transposable elements

Transposable elements (TEs) are DNA sequences that can autonomously change positions in a genome via various ‘cut-and-paste’ and ‘copy-and-paste’ mechanisms [68,69]. TEs can invade genomes even though they generally provide no adaptive advantage to the individual

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

carrying them [70–72]. To the contrary, new TE insertions in coding or regulatory sequences disrupt gene functions and cause deleterious effects in the host; only very rarely can specific insertions be co-opted to acquire novel, adaptive, functions for the host [72]. In sexual organisms, TEs can spread through panmictic populations because of their ability to rapidly colonize new genomes [10,73]. At the same time, sexual reproduction facilitates the purging of deleterious TE insertions, because recombination, segregation and genetic exchange among individuals improve the efficacy of selection [74,75]. In the absence of sex, TEs could therefore accumulate indefinitely, which led to the prediction that TEs could frequently drive the extinction of asexual lineages. Only asexual lineages without active TEs, or with efficient TE suppression mechanisms, would be able to persist over evolutionary times [75]. Consistent with this view, a study in bdelloid rotifers reported extremely low TE loads [76]. This prompted the authors to suggest that bdelloid rotifers could have been able to persist in the absence of sex for over 40 million years thanks to their largely TE-free genomes.

Our analysis of asexual animal genomes does not support the view that bdelloid rotifers have unusually low TE contents. The TE content of bdelloid rotifers (0.8% to 9.1%) is comparable to other asexual animal taxa (**Figure 4**), all of which are considerably younger than the bdelloids. Across the 24 genomes, there was large variation in total TE content, overall ranging from 6.6% to 17.9%, but with one species, the marbled crayfish, reaching 34.7%. Nevertheless, the abundance of TEs in asexual animal genomes appears to be generally lower than in sexual species, which range typically from 8.5-37.6% (median: 24.3%) [77]. Whether this difference is indeed driven by asexuality remains an open question as TE loads are known to be highly lineage-specific [20,78]. Furthermore, we annotated TEs in each genome via homology searches in general databases (see methods). This can result in an underestimation of TE loads relative to annotations based on species-specific TE libraries. However, this is unlikely to be of major concern in our study since the methods we used allowed us to identify more TEs than most of the individual genome studies (**Supplementary Table 3**). Specifically, most studies estimate TE loads from genome assemblies, which underestimates TE loads because regions with high repetitive contents are generally not assembled.

In addition to other lineage-specific characteristics, the cellular mechanisms underlying asexuality could also affect TE loads. For example, most forms of meiotic asexuality can allow for the purging of heterozygous TE-insertions, given the loss of heterozygosity between generations **(Box 2)**. Barring potential gene conversion events, this form of purging cannot occur under mitotic asexuality. However, in the genomes analyzed here, we did not find any effect of cellular mechanisms on TE loads (**Supplementary Figure 3**), likely

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

because the expected effect of the cellular mechanisms is very small relative to lineage-specific mechanisms. Moreover, host TE suppression mechanisms can contribute to the inactivation and subsequent degeneration of TE copies over time, independently of the cellular mechanism of asexuality [72,79].

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

Independently of the question of whether asexuality affects genome-level TE loads, our dataset allows us to study whether hybrid species have higher TE loads than non-hybrid species. Indeed, TE activity in hybrids is expected to be high because of mismatches between species-specific TEs and silencing machineries [13,15,81,82]. However, we do not find any difference in TE content according to hybrid vs intraspecific origin of asexuals (**Supplementary Figure 2**).

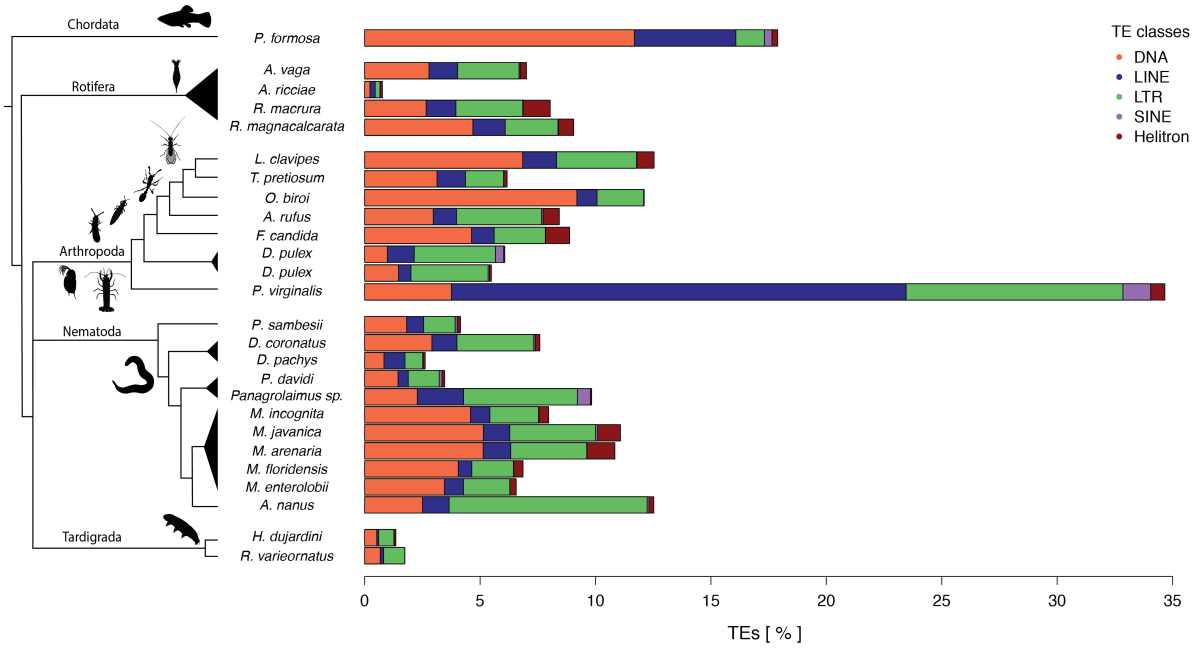


Figure 4: Percentage of transposable elements (TEs) in asexual genomes. Both the TE load

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

494 and frequency of TE classes vary substantially between individual asexual lineages. The TE
495 classes are: class I “cut-and-paste” DNA transposons (DNA), and class II “copy-and-paste”
496 long interspersed nuclear elements or autonomous non-LTR elements (LINEs), short
497 interspersed nuclear elements or non-autonomous non-LTR elements (SINEs), long terminal
498 repeat elements (LTR), and rolling-circle elements (Helitron).

499 Gene loss

500 Asexual animals are predicted to lose genes underlying sexual reproduction traits, including
501 male-specific traits and functions (e.g. male-specific organs, spermatogenesis), as well as
502 female traits involved in sexual reproduction (e.g., pheromone production, sperm storage
503 organs) [83]. In the absence of pleiotropic effects, gene loss is expected due to mutation
504 accumulation in the absence of purifying selection maintaining sexual traits, as well as to
505 directional selection to reduce costly sexual traits [84]. Some gene loss consistent with these
506 predictions is documented. For example, the sex determination genes xol-1 and tra-2 are
507 missing in the nematode *D. coronatus* [28]. Furthermore, genes believed to be involved in
508 male functions harbour an excess of deleterious mutations in the wasp *Leptopilina clavipes*
509 [19], which could represent the first step towards the loss of these genes. However, a similar
510 excess of deleterious mutations in genes with (presumed) male-specific functions was not
511 detected in the amazon molly *P. formosa* [16].
512
513 Species reproducing via mitotic asexuality are further predicted to lose genes specific to
514 meiotic processes [85]. The genes involved in meiosis have been studied in three of eight
515 mitotic parthenogens, as well as in *Rotaria* rotifers and *Diploscapter* nematodes, whose
516 cellular mechanisms of asexuality are unknown. Most meiotic genes have been found in the
517 four bdelloid rotifers [17,18] and in both species of *Diploscapter* nematodes [28,29]. There was also
518 no apparent loss of meiosis genes in the amazon molly *P. formosa* [16]. As much as the idea
519 is appealing, there does not seem to be any support for the predicted loss of meiotic genes
520 in mitotic asexuals. We note that the lack of our understanding of meiosis on the molecular
521 level outside of few model organisms (particularly yeast and *C. elegans*) makes the
522 interpretation of gene loss (or absence thereof) difficult. This is best illustrated by the fact
523 that losses of meiosis genes have also been reported in different sexual species, where
524 meiosis is clearly functional [86].
525
526 In summary, some gene loss consistent with the loss of different sexual functions has been
527 reported in several asexual species. However, a clear interpretation of gene loss in asexual
528 species is problematic because the function of the vast majority of genes is unknown in
529 these non-model organisms.

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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. It has also been proposed that HGTs represented an adaptive benefit which allows for the long term maintenance of asexuality [87]. Indeed, bdelloid rotifers have been reported to carry an unusually large amount (6.2% - 9.1%) of horizontally acquired genes compared to sexual lophotrochozoan genomes (0.08% - 0.7%) [18,88]. Many of these have contributed to adaptive divergence between bdelloid rotifer species [89]. However, there are no other ancient asexuals sequenced and evaluating the role of HGTs in the long-term persistence of asexuality is therefore not possible. In more recent asexuals, levels of HGT appear mostly low, e.g. in *Panagolaimus* (0.63% - 0.66%) and in two tardigrade species (0.8% - 0.97%)[18,30,37]. The only genome with a high reported fraction of HGT (2.8%) outside of the rotifers is the springtail *F. candida* [23]. This is a meiotic asexual, hence a relaxed constraint on chromosome pairing did not contribute to the high retention of horizontally acquired genes. Nevertheless, the presence of a gene for lignocellulose degradation in the springtail and in the root-knot nematode *M. incognita*, which was likely acquired via HGT in both species, supports an adaptive role of HGT in these asexuals [23,33]. However, such isolated events of adaptive HGTs are not specifically linked to asexuality, since they are reported in sexual species as well [90]. The potential relation of HGT and asexuality will remain unclear until we are able to reliably identify HGTs in more genomes of asexual as well as sexual species. Indeed, current reports of HGT are often unreliable because of the difficulty of distinguishing HGT from contamination [37].

Gene family expansions

Most genome papers, including those focussing on asexual animals, scan for expansions of specific gene families. Such expansions are then discussed in the light of the focal species' biology. The expansion of specific gene families *per se* is thus generally a species-specific trait [91] that is not related to asexuality. For example, expansions of stress response genes in *M. incognita* [33], *Panagrolaimus* spp. [30], and *R. varieornatus* [38] were suggested to be associated with the evolution of cryptobiosis in these species. To our knowledge, the only example of a gene family expansion that could be directly associated with asexuality is the diversification of the RNA silencing machinery of TEs in bdelloid rotifers [17]. TEs are expected to evolve reduced activity rates in asexual hosts (see section **Transposable elements**), and an improved RNA silencing machinery could be the mechanism underlying such reduced activity rates.

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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

577 Discussion

578 We re-analyzed 24 published genomes of asexual animals to investigate whether we can
579 detect genomic features that are characteristic of asexual animals in general. Many of the
580 original genome studies highlighted one or a few specific features in their focal asexual
581 species, and suggested that it might be linked to asexuality. However, our analyses and
582 review of published studies show that none of these genome features appear to be a general
583 consequence of asexuality given that none them was systematically replicated across even
584 a majority of analyzed species.
585
586 The variation among genomes of asexual species is at least in part due to species- or
587 lineage-specific traits. But variation among the features detected in the published single-
588 genome studies is also generated by differences in the methods used. Such differences are
589 often less obvious, and maybe less interesting to discuss, yet they can be critical in our
590 assessment of genome diversity among animals. In this work we thus re-analyzed several
591 key genome features with consistent methods. To minimize the effect of differences in
592 genome quality, we have used in priority robust methods, e.g. based on sequencing reads
593 rather than from assemblies. For example, re-estimating heterozygosity levels directly from
594 reads of each species allowed to show a strong effect of hybrid origin, but not of cellular
595 mechanism of asexuality (**Figure 2**). Another advantage of using the same methods over all
596 species is that it diminishes the "researcher degrees of freedom" [92–94]. For example, the
597 analysis of polyploid genomes requires choosing methods to call heterozygosity and ploidy.
598 By providing a common framework among species, we have shown that homoeolog
599 divergence is very diverse among polyploid asexuals.

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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 *Lineus* ribbon worms [34,54]. This rule applies more generally to all the species analysed with known transitions to asexuality, but it is important to highlight that all the non-hybrid species in our dataset are hexapods. Thus in principle the low heterozygosity could be a hexapod specific pattern, for example due to high gene conversion rates in hexapods. The taxonomic range of the sequenced species is wide but we are missing several clades rich in asexual species, such as mites or annelids [95,96]. These clades would be useful foci for future genomic studies of asexual species. Independently of the findings of such future studies, our results suggest that mitotic gene conversion (that acts independently of palindromes) plays a significant and highly underappreciated role in the evolution of asexual species of intraspecific origin. For example, it has been argued that one of the main benefits of sex could be the masking of recessive deleterious mutations (referred to as “complementation”) which would be exposed under many forms of meiotic asexuality [97,98]. If gene conversion is indeed pervasive, these arguments would extend to functionally mitotic forms of asexuality. Conversely, high rates of gene conversion could also allow for the purging of deleterious mutations while in the heterozygous state, as in highly selfing species (eg. [99,100]). Such purging could help explain why most of the genome scale studies did not find support for the theoretical expectation that asexual reproduction should result in increased rates of deleterious mutation accumulation (see section **Mutation accumulation and positive selection)**. More generally, given the major differences in genome evolution for asexuals of intra-specific vs. hybrid origin, our study calls for future theoretical approaches on the maintenance of sex that explicitly consider the loss *vs*. the maintenance of heterozygosity in asexuals.

In our evaluation of the general consequences of asexuality, we were not able to take two key aspects into account: survivor bias of asexual lineages, and characteristics of sexual ancestors. How often new asexual lineages emerge from sexual ancestors is completely unknown, but it has been speculated that in some taxa asexual lineages might emerge frequently, and then go extinct rapidly because of negative consequences of asexuality. In other words, asexuals that would exhibit the strongest consequences of asexuality, as predicted by theoretical models, are expected to go extinct the fastest. Such transient asexuals remain undetected in natural populations, because research focuses on asexual species or populations, and not on rare asexual females in sexual populations. Indeed, most of the species included in our study have persisted as asexuals for hundreds of thousands to

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

637 millions of years. They might thus be mostly representative of the subset of lineages that
638 suffer weaker consequences of asexuality. Finally, the key constraint for identifying
639 consequences of asexuality is that almost none of the published genome studies of asexual
640 animals included comparisons to close sexual relatives. This prevents the detection of
641 specific effects of asexuality, controlling for the variation among sexual species - which is
642 extensive for all of the genome features we analyzed and discussed in our study. Overall,
643 despite the importance of recombination rate variation for understanding the evolution of
644 sexual animal genomes (e.g., [101,102]), the genome-wide absence of recombination does not
645 appear to have the dramatic effects which are expected from classical theoretical models.
646 The reasons for this are probably a combination of lineage-specific patterns, differences
647 according to the origin of asexuality, and survivor bias of asexual lineages.

648 Methods

649 We combined different methods into a complete pipeline that collects published assemblies,
650 sequencing reads, and genome annotation data from online databases, and automatically
651 computes the genome features discussed here. The methods for the different steps in the
652 pipeline are detailed below. The pipeline is available at
653 https://github.com/KamilSJaron/genomic-features-of-asexual-animals. We used this pipeline
654 to gather and analyze the data for 29 sequenced individuals from 24 asexual species. For
655 some species, additional genomes to the ones we used were available, but we did not
656 include them because of low data quality and/or unavailable illumina reads (this was the
657 case for one sample of *M. incognita*, *M. floridensis* and multiple samples of *D. pulex* [24,33,36]).
658 Overall, the genome features computed were: ploidy, genome size, heterozygosity,
659 heterozygosity, haplotype divergence structure, transposable elements/ repeat content,
660 conserved gene content (see **Supplementary Text S3**), and palindrome abundance.
661
662 Core genome features (ploidy, haploid genome size, heterozygosity, repetitive fraction of the
663 genome, and characterisation of TE content) were estimated directly from sequencing reads
664 to avoid potential assembly biases in reference genome-based approaches. The raw reads
665 were publicly available for 27 samples and for three more samples shared by authors on
666 request. We cleaned the raw reads by removing adaptors and low quality bases using
667 Skewer (parameters "-z -m pe -n -q 26 -l 21") [103].
668
669 We used smudgeplot v0.1.3 (available at https://github.com/tbenavi1/smudgeplot) to
670 estimate ploidy levels. This method extracts from the read set unique kmer pairs that differ
671 by one SNP from each other. These kmer pairs are inferred to derive from heterozygous

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

672 genome regions. The sum of coverages of the kmer pairs is then compared against their
673 coverage ratio. This comparison separates different haplotype structures (**Supplementary**
674 **Figure 1b**). The most prevalent structure is then indicative of the overall ploidy of the
675 genome. We used this ploidy estimate in all species, except *A. vaga*. The most prevalent
676 structure suggested that this species is diploid. *A. vaga* is well characterized as tetraploid [58],
677 but we were unable to detect tetraploidy because homoeologs are too diverged to be
678 identified as such by the kmer-based smudgeplot method.
679
680 Using the inferred ploidy levels, we then estimated genome size and heterozygosity using an
681 extended version of GenomeScope [104]. GenomeScope estimates genome wide
682 heterozygosity via kmer spectra analysis, that is, by fitting a mixture model of evenly spaced
683 negative binomial distributions, where the number of fitted distributions is decided given the
684 input ploidy. Estimated distributions correspond to kmers that occur once, twice, etc., in the
685 genome. Fits are then used to estimate heterozygosity, the fraction of repeats in the
686 genome, as well as the 1n sequencing coverage. The latter is subsequently used for
687 estimation of genome size. The definition of heterozygosity for polyploids is not well
688 established (see **Box 3**), but GenomeScope distinguishes different types of heterozygous
689 loci in polyploids (as shown in **Figure 3**).
690
691 Kmer spectra analysis is affected by the choice of kmer length. Longer kmers require higher
692 sequencing coverage, but lead to more informative kmer spectra. We have chosen the
693 default kmer size 21 nt for all species except the marbled crayfish, where we chose kmer
694 length 17 nt due to low sequencing coverage.
695
696 We quantified transposable elements using DnaPipeTE v1.2 [105]. The method uses haploid
697 genome size (parameter -genome_size) to subsample sequencing reads to 0.5x coverage
698 (parameter -genome_coverage). Subsampled reads are then assembled using an assembler
699 that can deal with uneven coverages, and annotated using the database of known TEs. This
700 process is repeated three times (parameter -sample_number), and the union of results
701 represents the repeat library. Additionally, repeats are annotated as TEs if their sequence
702 matches known TEs by homology (for details see [105]). Our reported values of TE loads
703 include only repeats that were annotated as TEs, i.e., we did not include 'unknown' repeats
704 which consist of tandem repeats (satellite repeats), duplications or very divergent/unknown
705 TEs.
706
707 The palindrome analysis was based on genome assemblies and their published annotations,
708 from 27 samples of 22 species (annotations were not available for *D. pulex* and *A. rufus*).

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

We performed collinearity analysis using MCScanX (untagged version released 28.3.2013) [106], allowing even a single gene to form a collinear bloc (parameter -s) if there were fewer than 100 genes in between (parameter -m). The output was then filtered to contain only blocs on the same scaffold in a reverse order. Furthermore we filtered all the homologous gene pairs that have appeared on the same strand. All the remaining blocks are palindromes, blocs built of reverse complementary genes on the same scaffold. See **Supplementary Text S2** for more details.

References

1. Neiman, M., Lively, C. M. & Meirmans, S. Why sex? A pluralist approach revisited. *Trends Ecol. Evol.* **32,** 589–600 (2017).
2. Sharp, N. P. & Otto, S. P. Evolution of sex: Using experimental genomics to select among competing theories. *Bioessays* **38,** 751–757 (2016).
3. Bell, G. *The Masterpiece of Nature: The Evolution and Genetics of Sexuality*. (Univ of California Press, 1982).
4. Suomalainen, E., Saura, A. & Lokki, J. *Cytology and evolution in parthenogenesis*. (CRC Press, 1987).
5. Muller, H. J. The relation of recombination to mutational advance. *Mutat. Res.* **1,** 2–9 (1964).
6. Felsenstein, J. The evolutionary advantage of recombination. *Genetics* **78,** 737–756 (1974).
7. Keightley, P. D. & Otto, S. P. Interference among deleterious mutations favours sex and recombination in finite populations. *Nature* **443,** 89–92 (2006).
8. Birky, C. W., Jr. Heterozygosity, heteromorphy, and phylogenetic trees in asexual eukaryotes. *Genetics* **144,** 427–437 (1996).
9. Balloux, F., Lehmann, L. & de Meeûs, T. The population genetics of clonal and partially clonal diploids. *Genetics* **164,** 1635–1644 (2003).
10. Hickey, D. A. Selfish DNA: a sexually-transmitted nuclear parasite. *Genetics* **101,** 519–531 (1982).

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

11. Brandt, A. *et al.* Effective purifying selection in ancient asexual oribatid mites. *Nat. Commun.* **8,** 873 (2017).
12. Fontaneto, D., Tang, C. Q., Obertegger, U., Leasi, F. & Barraclough, T. G. Different diversification rates between sexual and asexual organisms. *Evol. Biol.* **39,** 262–270 (2012).
13. Arkhipova, I. R. & Rodriguez, F. Genetic and epigenetic changes involving (retro)transposons in animal hybrids and polyploids. *Cytogenet. Genome Res.* **140,** 295–311 (2013).
14. Neiman, M., Sharbel, T. F. & Schwander, T. Genetic causes of transitions from sexual reproduction to asexuality in plants and animals. *J. Evol. Biol.* **27,** 1346–1359 (2014).
15. Rodriguez, F. & Arkhipova, I. R. Transposable elements and polyploid evolution in animals. *Curr. Opin. Genet. Dev.* **49,** 115–123 (2018).
16. Warren, W. C. *et al.* Clonal polymorphism and high heterozygosity in the celibate genome of the Amazon molly. *Nat Ecol Evol* **2,** 669–679 (2018).
17. Flot, J.-F. *et al.* Genomic evidence for ameiotic evolution in the bdelloid rotifer Adineta vaga. *Nature* **500,** 453–457 (2013).
18. Nowell, R. W. *et al.* Comparative genomics of bdelloid rotifers: Insights from desiccating and nondesiccating species. *PLoS Biol.* **16,** e2004830 (2018).
19. Kraaijeveld, K. *et al.* Decay of sexual trait genes in an asexual parasitoid wasp. *Genome Biol. Evol.* **8,** 3685–3695 (2016).
20. Bast, J. *et al.* No accumulation of transposable elements in asexual arthropods. *Mol. Biol. Evol.* **33,** 697–706 (2016).
21. Lindsey, A. R. I. *et al.* Comparative genomics of the miniature wasp and pest control agent Trichogramma pretiosum. *BMC Biol.* **16,** 54 (2018).
22. Oxley, P. R. *et al.* The genome of the clonal raider ant Cerapachys biroi. *Curr. Biol.* **24,** 451–458 (2014).
23. Faddeeva-Vakhrusheva, A. *et al.* Coping with living in the soil: the genome of the parthenogenetic springtail Folsomia candida. *BMC Genomics* **18,** 493 (2017).

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

765 24. Tucker, A. E., Ackerman, M. S., Eads, B. D., Xu, S. & Lynch, M. Population-genomic
766 insights into the evolutionary origin and fate of obligately asexual Daphnia pulex. *Proc.*
767 *Natl. Acad. Sci. U. S. A.* **110,** 15740–15745 (2013).
768 25. Xu, S., Omilian, A. R. & Cristescu, M. E. High rate of large-scale hemizygous deletions
769 in asexually propagating Daphnia: implications for the evolution of sex. *Mol. Biol. Evol.*
770 **28,** 335–342 (2011).
771 26. Jiang, X., Tang, H., Ye, Z. & Lynch, M. Insertion polymorphisms of mobile genetic
772 elements in sexual and asexual populations of Daphnia pulex. *Genome Biol. Evol.*
773 (2017). doi:10.1093/gbe/evw302
774 27. Gutekunst, J. *et al.* Clonal genome evolution and rapid invasive spread of the marbled
775 crayfish. *Nat Ecol Evol* **2,** 567–573 (2018).
776 28. Hiraki, H. *et al.* Genome analysis of Diploscapter coronatus: insights into molecular
777 peculiarities of a nematode with parthenogenetic reproduction. *BMC Genomics* **18,** 478
778 (2017).
779 29. Fradin, H. *et al.* Genome architecture and evolution of a unichromosomal asexual
780 nematode. *Curr. Biol.* **27,** 2928–2939.e6 (2017).
781 30. Schiffer, P. H. *et al.* Signatures of the evolution of parthenogenesis and cryptobiosis in
782 the genomes of panagrolaimid nematodes. *bioRxiv* 159152 (2017). doi:10.1101/159152
783 31. Schiffer, P. H. *et al.* The gene regulatory program of Acrobeloides nanus reveals
784 conservation of phylum-specific expression. *Proc. Natl. Acad. Sci. U. S. A.* **115,** 4459–
785 4464 (2018).
786 32. Blanc-Mathieu, R. *et al.* Hybridization and polyploidy enable genomic plasticity without
787 sex in the most devastating plant-parasitic nematodes. *PLoS Genet.* **13,** e1006777
788 (2017).
789 33. Abad, P. *et al.* Genome sequence of the metazoan plant-parasitic nematode
790 Meloidogyne incognita. *Nat. Biotechnol.* **26,** 909–915 (2008).
791 34. Szitenberg, A. *et al.* Comparative genomics of apomictic root-knot nematodes:
792 hybridization, ploidy, and dynamic genome change. *Genome Biol. Evol.* **9,** 2844–2861

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

(2017).

35. Szitenberg, A. *et al.* Genetic Drift, Not Life History or RNAi, Determine Long-Term Evolution of Transposable Elements. *Genome Biol. Evol.* **8,** 2964–2978 (2016).
36. Lunt, D. H., Kumar, S., Koutsovoulos, G. & Blaxter, M. L. The complex hybrid origins of the root knot nematodes revealed through comparative genomics. *PeerJ* **2,** e356 (2014).
37. Koutsovoulos, G. *et al.* No evidence for extensive horizontal gene transfer in the genome of the tardigrade Hypsibius dujardini. *Proc. Natl. Acad. Sci. U. S. A.* **113,** 5053–5058 (2016).
38. Hashimoto, T. *et al.* Extremotolerant tardigrade genome and improved radiotolerance of human cultured cells by tardigrade-unique protein. *Nat. Commun.* **7,** 12808 (2016).
39. Cavalier-Smith, T. Origins of the machinery of recombination and sex. *Heredity* **88,** 125–141 (2002).
40. Schultz, R. J. Unisexual fish: laboratory synthesis of a 'species'. *Science* **179,** 180–181 (1973).
41. van der Kooi, C. J., Matthey-Doret, C. & Schwander, T. Evolution and comparative ecology of parthenogenesis in haplodiploid arthropods. *Evol Lett* **1,** 304–316 (2017).
42. Engelstädter, J. Asexual but not clonal: evolutionary processes in automictic populations. *Genetics* **206,** 993–1009 (2017).
43. Lenormand, T., Engelstädter, J., Johnston, S. E., Wijnker, E. & Haag, C. R. Evolutionary mysteries in meiosis. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **371,** (2016).
44. Melters, D. P., Paliulis, L. V., Korf, I. F. & Chan, S. W. L. Holocentric chromosomes: convergent evolution, meiotic adaptations, and genomic analysis. *Chromosome Res.* **20,** 579–593 (2012).
45. Cifuentes, M., Grandont, L., Moore, G., Chèvre, A. M. & Jenczewski, E. Genetic regulation of meiosis in polyploid species: new insights into an old question. *New Phytol.* **186,** 29–36 (2010).
46. Schurko, A. M., Neiman, M. & Logsdon, J. M. Signs of sex: what we know and how we

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

know it. *Trends Ecol. Evol.* **24,** 208–217 (2009).

47. Neiman, M., Meirmans, S. & Meirmans, P. G. What can asexual lineage age tell us about the maintenance of sex? *Ann. N. Y. Acad. Sci.* **1168,** 185–200 (2009).
48. Hill, W. G. & Robertson, A. The effect of linkage on limits to artificial selection. *Genet. Res.* **8,** 269–294 (1966).
49. Fisher, R. A. *The genetical theory of natural selection*. **154,** (Clarendon Press, 1930).
50. Muller, H. J. Some genetic aspects of sex. *Am. Nat.* 118–138 (1932).
51. Neiman, M., Meirmans, P. G., Schwander, T. & Meirmans, S. Sex in the wild: How and why field-based studies contribute to solving the problem of sex. *Evolution* **72,** 1194–1203 (2018).
52. Bast, J. *et al.* Consequences of asexuality in natural populations: insights from stick insects. *Mol. Biol. Evol.* **35,** 1668–1677 (2018).
53. Hollister, J. D. *et al.* Recurrent loss of sex is associated with accumulation of deleterious mutations in Oenothera. *Mol. Biol. Evol.* **32,** 896–905 (2015).
54. Ament-Velásquez, S. L. *et al.* Population genomics of sexual and asexual lineages in fissiparous ribbon worms (Lineus, Nemertea): hybridization, polyploidy and the Meselson effect. *Mol. Ecol.* **25,** 3356–3369 (2016).
55. Schön, I., Martens, K. & Dijk, P. van. *Lost sex: the evolutionary biology of parthenogensis*. (Springer, 2009).
56. Hartl, D. L. & Clark, A. G. *Principles of population genetics*. **116,** (Sinauer associates Sunderland, 1997).
57. Glover, N. M., Redestig, H. & Dessimoz, C. Homoeologs: what are they and how do we infer them? *Trends Plant Sci.* **21,** 609–621 (2016).
58. Mark Welch, D. B., Mark Welch, J. L. & Meselson, M. Evidence for degenerate tetraploidy in bdelloid rotifers. *Proc. Natl. Acad. Sci. U. S. A.* **105,** 5145–5149 (2008).
59. Weir, W. *et al.* Population genomics reveals the origin and asexual evolution of human infective trypanosomes. *Elife* **5,** 1–14 (2016).
60. Rozen, S. *et al.* Abundant gene conversion between arms of palindromes in human and

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

849 ape Y chromosomes. *Nature* **423,** 873–876 (2003).

850 61. Marais, G. A. B., Campos, P. R. A. & Gordo, I. Can intra-Y gene conversion oppose the
851 degeneration of the human Y chromosome? A simulation study. *Genome Biol. Evol.* **2,**
852 347–357 (2010).

853 62. Trombetta, B. & Cruciani, F. Y chromosome palindromes and gene conversion. *Hum.*
854 *Genet.* **136,** 605–619 (2017).

855 63. Fan, S. & Meyer, A. Evolution of genomic structural variation and genomic architecture
856 in the adaptive radiations of African cichlid fishes. *Front. Genet.* **5,** 163 (2014).

857 64. Chen, L., Chamberlain, A. J., Reich, C. M., Daetwyler, H. D. & Hayes, B. J. Detection
858 and validation of structural variations in bovine whole-genome sequence data. *Genet.*
859 *Sel. Evol.* **49,** 13 (2017).

860 65. Hum, Y. F. & Jinks-Robertson, S. Mitotic gene conversion tracts associated with repair
861 of a defined double-strand break in Saccharomyces cerevisiae. *Genetics* **207,** 115–128
862 (2017).

863 66. Lee, P. S. *et al.* A fine-structure map of spontaneous mitotic crossovers in the yeast
864 Saccharomyces cerevisiae. *PLoS Genet.* **5,** e1000410 (2009).

865 67. Keith, N. *et al.* High mutational rates of large-scale duplication and deletion in Daphnia
866 pulex. *Genome Res.* **26,** 60–69 (2016).

867 68. Wicker, T., Sabot, F., Hua-Van, A. & Bennetzen, J. L. A unified classification system for
868 eukaryotic transposable elements. *Nat. Rev. Genet.* **8,** 973–982 (2007).

869 69. Burt, A. & Trivers, R. *Genes in conflict: the biology of selfish genetic elements*.
870 (Belknapp Press, 2006).

871 70. Doolittle, W. F. & Sapienza, C. Selfish genes, the phenotype paradigm and genome
872 evolution. *Nature* **284,** 601–603 (1980).

873 71. Le Rouzic, A., Boutin, T. S. & Capy, P. Long-term evolution of transposable elements.
874 *Proc. Natl. Acad. Sci. U. S. A.* **104,** 19375–19380 (2007).

875 72. Hua-Van, A., Le Rouzic, A., Boutin, T. S., Filée, J. & Capy, P. The struggle for life of the
876 genome's selfish architects. *Biol. Direct* **6,** 19 (2011).

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

877 73. Zeyl, C., Bell, G. & Green, D. M. Sex and the spread of retrotransposon Ty3 in
878 experimental populations of Saccharomyces cerevisiae. *Genetics* **143,** 1567–1577
879 (1996).
880 74. Nuzhdin, S. V. & Petrov, D. A. Transposable elements in clonal lineages: lethal
881 hangover from sex. *Biol. J. Linn. Soc. Lond.* **79,** 33–41 (2003).
882 75. Wright, S. & Finnegan, D. Genome evolution: Sex and the transposable element. *Curr.*
883 *Biol.* **11,** R296–R299 (2001).
884 76. Arkhipova, I. & Meselson, M. Transposable elements in sexual and ancient asexual
885 taxa. *Proc. Natl. Acad. Sci. U. S. A.* **97,** 14473–14477 (2000).
886 77. Canapa, A., Barucca, M., Biscotti, M. A., Forconi, M. & Olmo, E. Transposons, genome
887 size, and evolutionary insights in animals. *Cytogenet. Genome Res.* **147,** 217–239
888 (2015).
889 78. Kofler, R., Betancourt, A. J. & Schlötterer, C. Sequencing of pooled DNA samples (Pool-
890 Seq) uncovers complex dynamics of transposable element insertions in Drosophila
891 melanogaster. *PLoS Genet.* **8,** e1002487 (2012).
892 79. Aravin, A. a., Hannon, G. J. & Brennecke, J. The Piwi-piRNA pathway provides an
893 adaptive defense in the transposon arms race. *Science* **318,** 761–764 (2007).
894 80. Agren, J. A., Greiner, S., Johnson, M. T. & Wright, S. I. No evidence that sex and
895 transposable elements drive genome size variation in evening primroses. *Evolution* **69,**
896 1053–1062 (2015).
897 81. Agren, J. A. & Wright, S. I. Co-evolution between transposable elements and their
898 hosts: a major factor in genome size evolution? *Chromosome Res.* **19,** 777–786 (2011).
899 82. Romero-Soriano, V. *et al.* Transposable element misregulation is linked to the
900 divergence between parental piRNA pathways in Drosophila hybrids. *Genome Biol.*
901 *Evol.* **9,** 1450–1470 (2017).
902 83. van der Kooi, C. J. & Schwander, T. On the fate of sexual traits under asexuality. *Biol.*
903 *Rev. Camb. Philos. Soc.* **89,** 805–819 (2014).
904 84. Schwander, T., Crespi, B. J., Gries, R. & Gries, G. Neutral and selection-driven decay of

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

905 sexual traits in asexual stick insects. *Proc. Biol. Sci.* **280,** 20130823 (2013).
906 85. Schurko, A. M. & Logsdon, J. M. Using a meiosis detection toolkit to investigate ancient
907 asexual 'scandals' and the evolution of sex. *Bioessays* **30,** 579–589 (2008).
908 86. Tvedte, E. S., Forbes, A. A. & Logsdon, J. M., Jr. Retention of core meiotic genes
909 across diverse Hymenoptera. *J. Hered.* **108,** 791–806 (2017).
910 87. Danchin, E. G. J. *et al.* Multiple lateral gene transfers and duplications have promoted
911 plant parasitism ability in nematodes. *Proc. Natl. Acad. Sci. U. S. A.* **107,** 17651–17656
912 (2010).
913 88. Gladyshev, E. A., Meselson, M. & Arkhipova, I. R. Massive horizontal gene transfer in
914 bdelloid rotifers. *Science* **320,** 1210–1213 (2008).
915 89. Boschetti, C. *et al.* Biochemical Diversification through Foreign Gene Expression in
916 Bdelloid Rotifers. *PLoS Genet.* **8,** e1003035 (2012).
917 90. Boto, L. Horizontal gene transfer in the acquisition of novel traits by metazoans. *Proc.*
918 *Biol. Sci.* **281,** 20132450 (2014).
919 91. Lespinet, O., Wolf, Y. I., Koonin, E. V. & Aravind, L. The role of lineage-specific gene
920 family expansion in the evolution of eukaryotes. *Genome Res.* **12,** 1048–1059 (2002).
921 92. Wallach, J. D., Boyack, K. W. & Ioannidis, J. P. A. Reproducible research practices,
922 transparency, and open access data in the biomedical literature, 2015-2017. *PLoS Biol.*
923 **16,** e2006930 (2018).
924 93. Simmons, J. P., Nelson, L. D. & Simonsohn, U. False-positive psychology: undisclosed
925 flexibility in data collection and analysis allows presenting anything as significant.
926 *Psychol. Sci.* **22,** 1359–1366 (2011).
927 94. Gelman, A. & Loken, E. The garden of forking paths: Why multiple comparisons can be
928 a problem, even when there is no 'fishing expedition' or 'p-hacking' and the research
929 hypothesis was posited ahead of time. (2013).
930 95. Veresoglou, S. D., Halley, J. M. & Rillig, M. C. Extinction risk of soil biota. *Nat. Commun.*
931 **6,** 8862 (2015).
932 96. Norton, R. A. & Palmer, S. C. The distribution, mechanisms and evolutionary

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

significance of parthenogenesis in oribatid mites. in *The Acari: Reproduction, development and life-history strategies* (eds. Schuster, R. & Murphy, P. W.) 107–136 (Springer Netherlands, 1991).

97. Archetti, M. Complementation, genetic conflict, and the evolution of sex and recombination. *J. Hered.* **101,** 21–33 (2010).
98. Archetti, M. Recombination and loss of complementation: a more than two-fold cost for parthenogenesis. *J. Evol. Biol.* **17,** 1084–1097 (2004).
99. Szövényi, P. *et al.* Efficient purging of deleterious mutations in plants with haploid selfing. *Genome Biol. Evol.* **6,** 1238–1252 (2014).
100. Glémin, S. & Ronfort, J. Adaptation and maladaptation in selfing and outcrossing species: new mutations versus standing variation. *Evolution* **67,** 225–240 (2013).
101. Stapley, J., Feulner, P. G. D., Johnston, S. E., Santure, A. W. & Smadja, C. M. Recombination: the good, the bad and the variable. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **372,** (2017).
102. Bachtrog, D. Y-chromosome evolution: emerging insights into processes of Y-chromosome degeneration. *Nat. Rev. Genet.* **14,** 113–124 (2013).
103. Jiang, H., Lei, R., Ding, S.-W. & Zhu, S. Skewer: a fast and accurate adapter trimmer for next-generation sequencing paired-end reads. *BMC Bioinformatics* **15,** 182 (2014).
104. Vurture, G. W. *et al.* GenomeScope: fast reference-free genome profiling from short reads. *Bioinformatics* **33,** 2202–2204 (2017).
105. Goubert, C. *et al.* De novo assembly and annotation of the Asian tiger mosquito (Aedes albopictus) repeatome with dnaPipeTE from raw genomic reads and comparative analysis with the yellow fever mosquito (Aedes aegypti). *Genome Biol. Evol.* **7,** 1192–1205 (2015).
106. Wang, Y. *et al.* MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Res.* **40,** e49 (2012).
107. Handoo, Z. A. *et al.* Morphological, molecular, and differential-host characterization of Meloidogyne floridensis n. sp. (Nematoda: Meloidogynidae), a root-knot nematode

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

parasitizing peach in Florida. *J. Nematol.* **36,** 20–35 (2004).

108. Lahl, V., Sadler, B. & Schierenberg, E. Egg development in parthenogenetic nematodes: variations in meiosis and axis formation. *Int. J. Dev. Biol.* **50,** 393–398 (2006).

109. Waterhouse, R. M. *et al.* BUSCO applications from quality assessments to gene prediction and phylogenomics. *Mol. Biol. Evol.* **35,** 543–548 (2017).

Acknowledgments: We would like to thank Philipp H. Schiffer, Jean-Francois Flot, Reuben W. Nowell, Yuko Ulrich, Dave Lunt, Julian Gutekunst and Mark Blaxter for comments and discussions. We would also like to thank all the authors who deposited their data in public databases with correct metadata and all the authors who were helpful when we asked for data or clarifications.

Funding: This study was supported by funding from Swiss SNF grants PP00P3_170627 and CRSII3_160723.

Author contributions: K.S.J. and T.S. conceived the study, K.S.J. and J.B. collected the data; K.S.J. and T.R.R.B. performed the analyses; K.S.J., J.B. and T.S. reviewed the literature; K.S.J., T.S., J.B. and M.R.R. wrote the manuscript; All authors were involved in discussions about results and interpretations.

Competing interests: None declared.

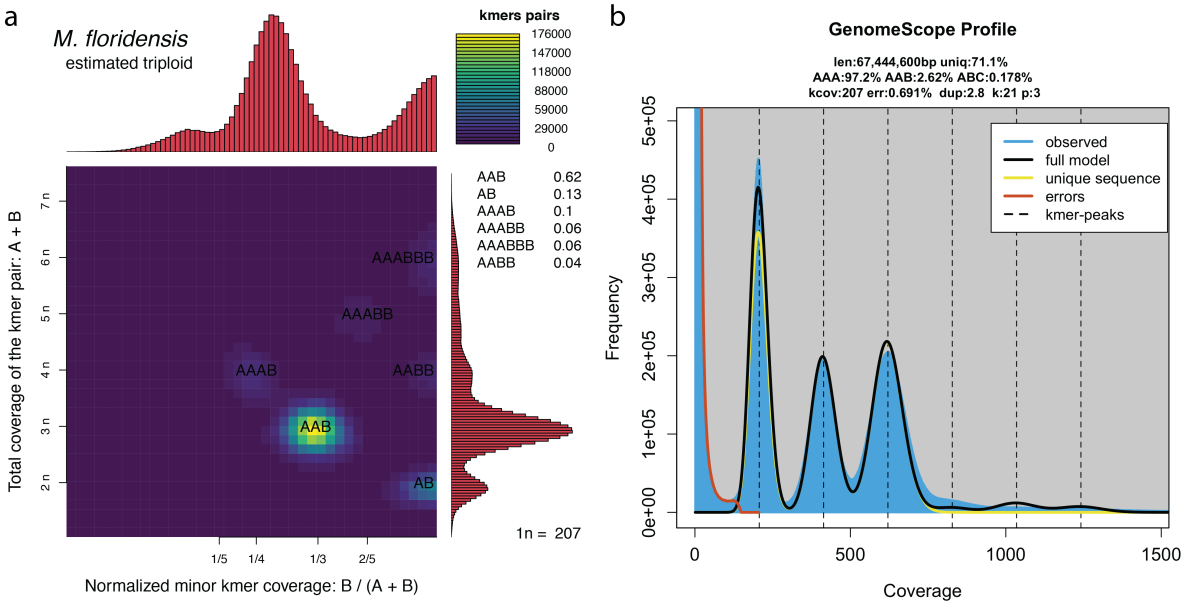
Supplementary materials

S1 Ploidy and reproductive mode of *M. floridensis*

The nematode *M. floridensis* was reported as a diploid species with a mechanism of asexuality functionally equivalent to terminal fusion (absence of the 2nd meiotic division), based on cytological analyses [107]. Our analyses indicate that *M. floridensis* is triploid rather than diploid (**Supplementary Figure 1**), and the heterozygosity detected in our and previous studies [107] is inconsistent with classical terminal fusion (which should result in largely homozygous genomes, see Box 2 and **Figure 2**). Terminal fusion can be associated with high heterozygosity under inverted meiosis (which is most likely the case in nematodes of the genus *Acrobeloides* [108]. However, inverted meiosis in *M. floridensis* is rather unlikely given that all other meiotic species in the genus have regular meiosis. We therefore believe that the study of Handoo et al is either based on an unusual *M. floridensis* strain that has not been used in any genome study thus far or that the cytology inferred by Handoo et al is not

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

correct. These interpretations are further supported by the fact that Handoo et al report on analyses of large numbers of males of *M. floridensis*, while males are unknown/unusual for the strains used in the genome studies. Unfortunately, it is impossible to evaluate the evidence that supported diploidy and terminal fusion in *M. floridensis* as the study by Handoo et al does not include pictures of karyotypes or egg cells (which is very unusual for this type of research). Given the genomic evidence is very clear, we consider *M. floridensis* to be triploid for all our analyses and the cellular mechanism of asexuality as "unknown".



Supplementary Figure 1: Genomic evidence of triploidy in *M. floridensis*. a | the smudgeplot shows dominance of a triploid (AAB) genome structure. The smudges corresponding to higher ploïdies are likely originating from paralogs. The diploid kmer pairs (AB) represent situations where the third allele is diverged from the two more than one nucleotide. **b |** kmer spectra analysis of *M. floridensis* shows a typical triploid genome structure with haploid, diploid and triploid peaks and expected distances from each other.

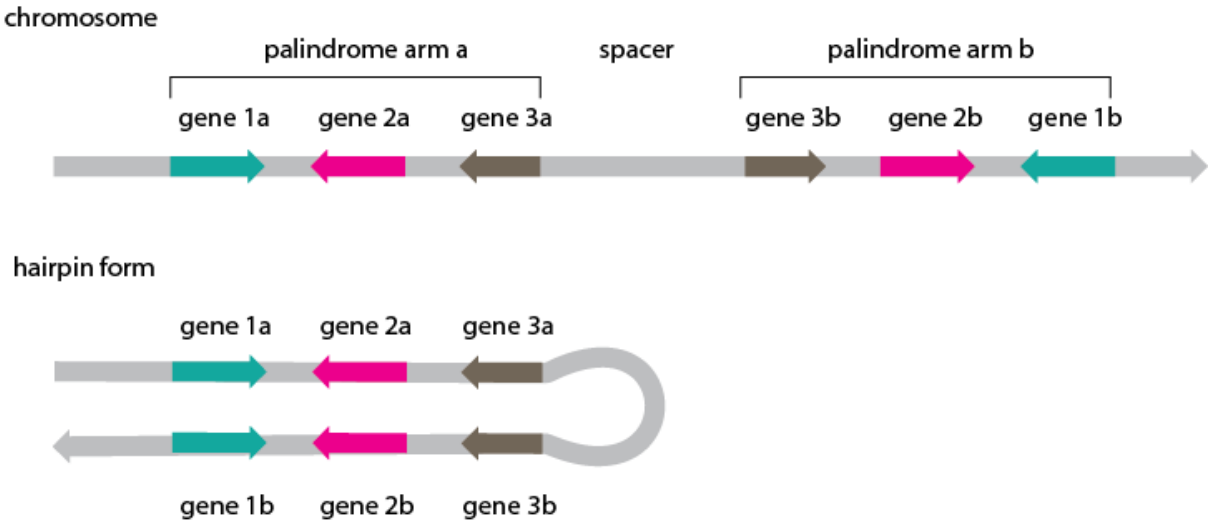
S2 Palindrome detection

Palindromes are formed by two homologous reverse complementary sequences on the same chromosome (**Supplementary Figure 2**). Palindromes can facilitate gene conversion and therefore help to escape mutational meltdown via Muller's ratchet [61,62]. To test if they play such a role in asexual organisms we identify palindromes using colinearity analysis imlplemented in program MCScanX [106]. The default parameters of the software (used in the genome studies of asexual species, personal communication of the authors of [17,23]) define a collinear block as a sequence of at least 5 genes that are no more than 25 genes apart from each other and then search for such blocks with palindromic arrangement. We have reanalysed the genomes allowing for short palindromes of a single gene because a

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

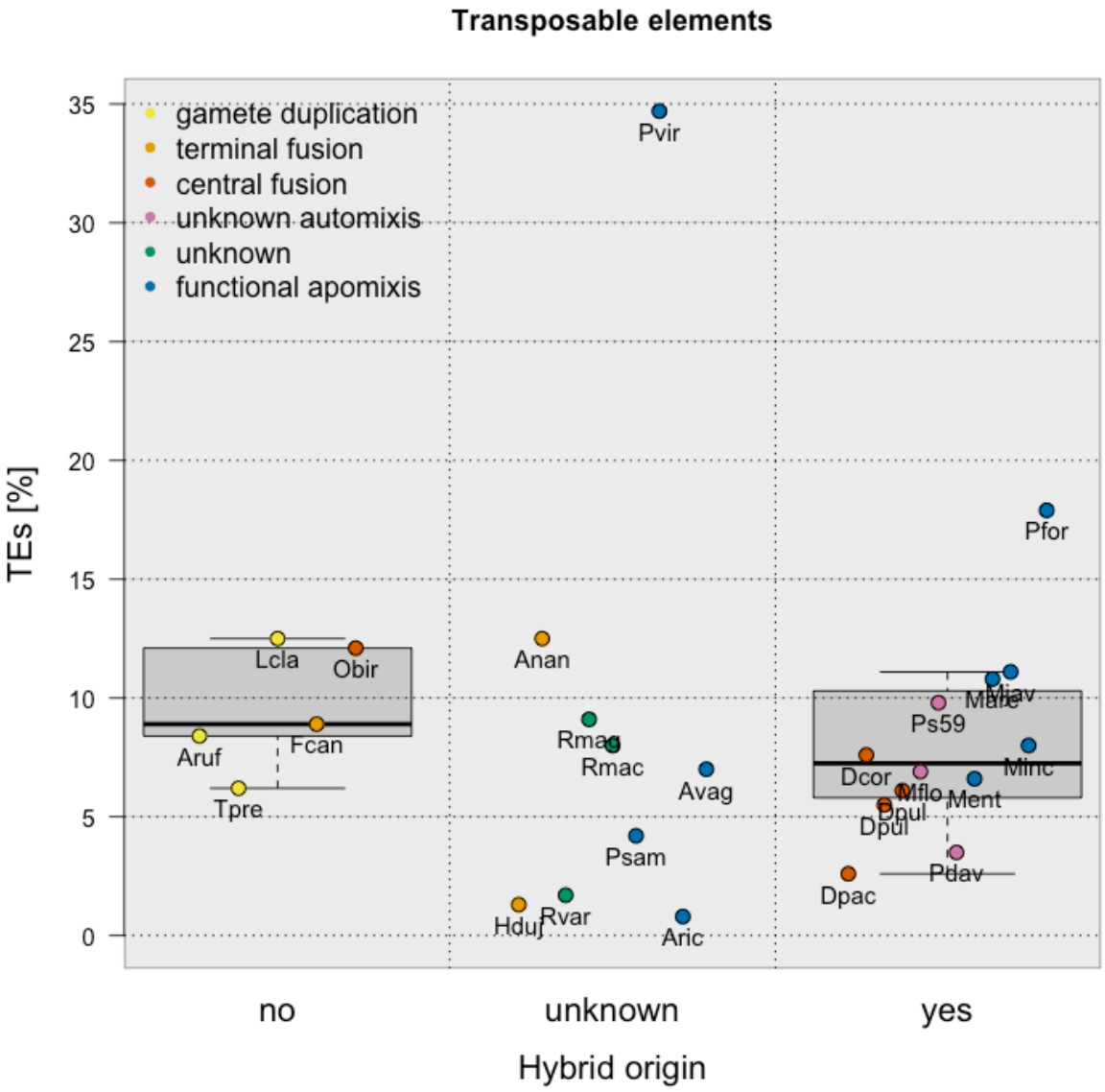
palindrome could carry fewer than five genes and still be biologically relevant. Detected collinear blocks were filtered to contain only reverse complementary collinear blocks on the same chromosome, since only such structures have the capacity to form a hairpin (**Supplementary Figure 2**).

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. (cit).



Supplementary Figure 2: 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.

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.



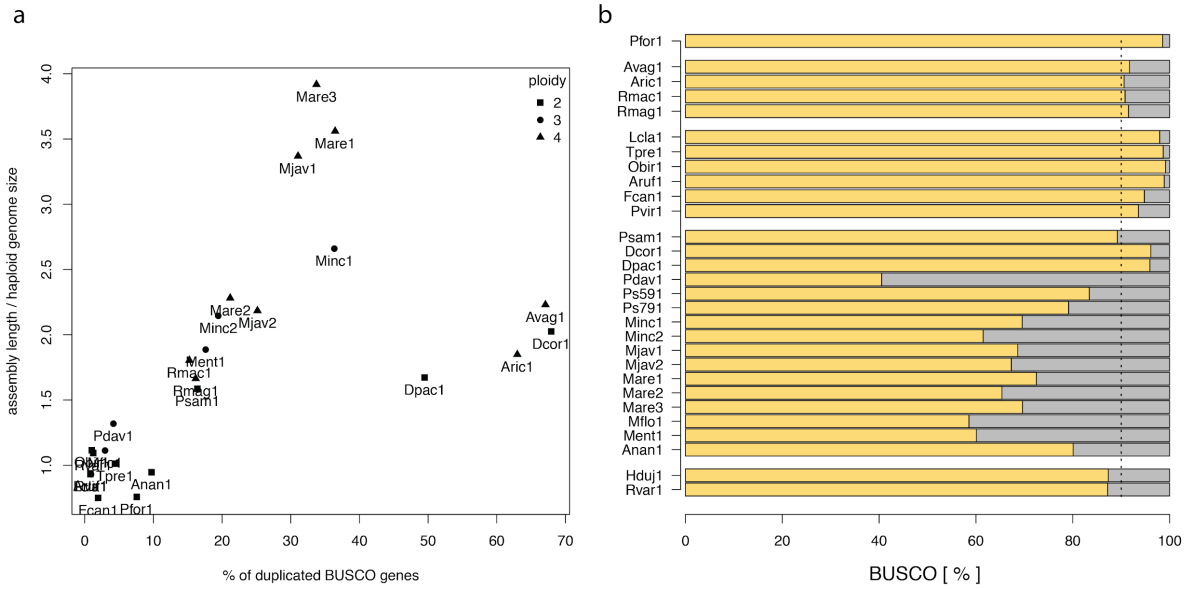
Supplementary Figure 3: Transposable elements with respect to reproduction mode and hybrid origin. Neither hybrid origin (p-value = 0.36) nor cellular mechanism of asexuality (p-value = 0.84) are strong drivers of the TE content in asexual animals.

S3 Conserved gene content

We aimed to provide insights into gene duplications and losses by quantifying conserved single copy orthologs (BUSCO genes) [109]. BUSCO genes are defined as a set of genes that are present as a single copy in at least 90% of species inventoried in a curated database. All of the species used to build this database are sexual, and we initially hypothesised that both higher duplication rates and gene losses in asexual as compared to sexual species could be

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

reflected in the percentages of missing and duplicated BUSCO genes in the analyzed asexual genomes. However, organisms that are highly heterozygous are prone to separate assembly of homologous haplotypes. In such split genome assemblies, BUSCO genes will falsely appear to be duplicated. To investigate whether split haplotype assemblies are of concern in the analyzed asexual genomes, we deduced the level of haplotype splitting in the assembled genomes by dividing the length of each assembly by the haploid genome size estimated from the read data with genomescope (higher frequencies of separate haplotype assemblies result in higher assembly length to haploid genome size ratios). We indeed found that BUSCO genes appear to be duplicated in genome assemblies consisting of split haplotypes, with the highest level of "artificial duplication" found in polyploid species of hybrid origin (**Supplementary Figure 4a**).



Supplementary Figure 4: Conserved single copy orthologs. a | the fraction of duplicated BUSCO genes is correlated to the ratio of assembly length to haploid genome size. **b** l 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.

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

bioRxiv preprint doi: https://doi.org/10.1101/497495; this version posted December 17, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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