Genomic features of asexual animals

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

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

Abstract

Evolution under asexuality is predicted to impact genomes in numerous ways, but empirical evidence remains unclear. Case studies of individual asexual animals have reported peculiar genomic features which 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.

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

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.

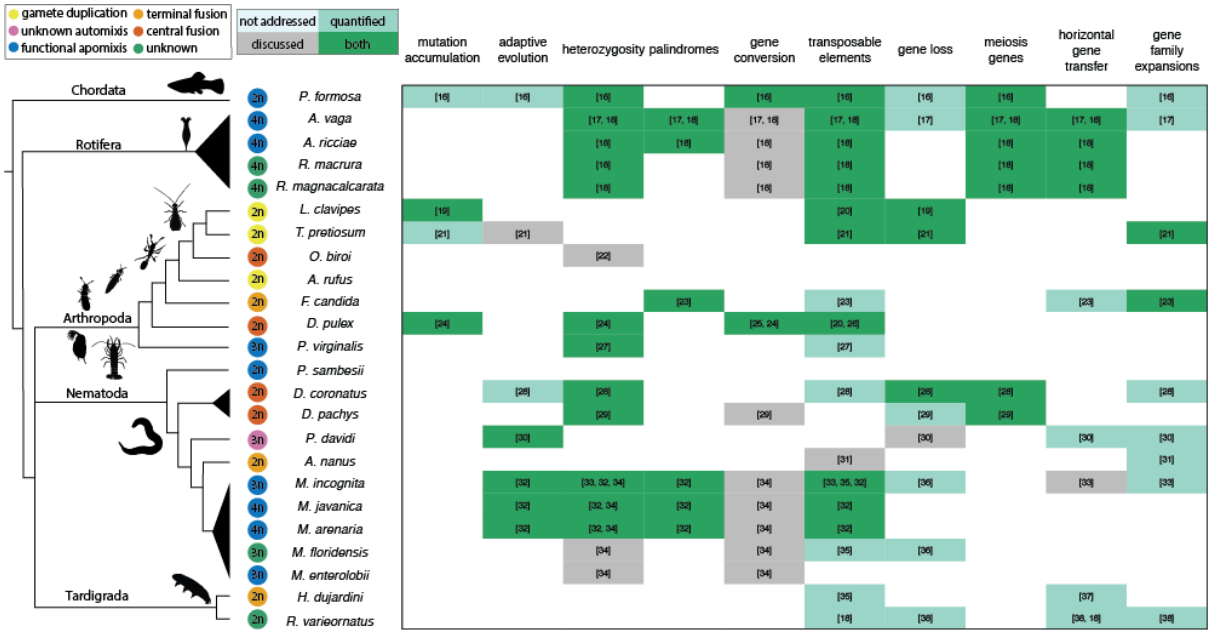


Figure 1: **Genome features studied in asexual animal species.** The phylogeny displays the taxonomic relation of the 24 sequenced asexual animal species considered here. The color of the circle indicates the cellular mechanism of asexuality and the number inside the

95 circle the ploidy of the species (see Supplemental Table 1 for details). Note that *M.*
96 *floridensis* is considered 2n in the published genome studies but our analyses clearly show
97 that this species is triploid (Supplementary Materials). The 24 species correspond to at least
98 16 independent transitions from sexual to asexual reproduction; species that might derive
99 from the same original transition are grouped in triangles. Each original genome article
100 explored a given set of genome features: the green cells (both light and dark) represent
101 cases where the genomic feature was quantified; the dark cells (grey, dark green) represent
102 studies where the genomic features were discussed with respect to asexuality.
103 Heterozygosity, palindromes and transposable elements were reanalysed in this study, the
104 discussion of the remaining features is based on the analyses reported in the individual
105 genome studies [16–38].
106
107 **Box 1: Transitions to asexuality**
108 Meiotic sex evolved once ~1.5 billion years ago, and since then remained the predominant
109 mode of reproduction in eukaryotes [3,39]. Current asexual animals therefore derive from a
110 sexual ancestor, but how transitions from sexual to asexual reproduction occur can vary.
111 While the molecular changes underlying different types of transitions are unknown, the
112 expected genomic consequences of asexuality vary extensively among them [14].
113 **Hybrid origin**. Hybridization between sexual species can generate hybrid females that
114 reproduce asexually [14,40]. Asexuality caused by hybridization generates high levels of
115 heterozygosity, corresponding to the divergence between the parental sexual species prior
116 to hybridization. Hybridization can also result in a burst of transposable element activity [13].
117 **Endosymbiont infection.** Infection with intracellular endosymbionts (such as *Wolbachia*,
118 *Cardinium* or *Rickettsia*) can cause asexuality, a pattern that frequent in species with
119 haplodiploid sex determination [41]. This type of transition often (but not always) results in fully
120 homozygous lineages because asexuality induction frequently occurs via gamete duplication
121 (see Box 2).
122 **Spontaneous mutations/Contagious asexuality.** Spontaneous mutations can also
123 underlie transitions from sexual to asexual reproduction. In addition, asexual females of
124 some species produce males that mate with females of sexual lineages, and thereby
125 generate new asexual strains (contagious asexuality). In both cases, the genomes of
126 incipient asexual lineages are expected to be very similar to those of their sexual relatives
127 and subsequent changes should be largely driven by the cellular mechanism underlying
128 asexuality (Box 2).
129
130 **Box 2: Cellular mechanisms of asexuality**

131 In sexual species offspring is generated through the fusion of male and female gametes. In
132 asexuals, females generate diploid (or polyploid) offspring from unfertilized oocytes via
133 different cellular mechanisms. The cellular mechanism used is predicted to affect genome
134 evolution and especially heterozygosity levels. For details see [4,42].
135 **Mitotic asexuality** (Apomixis). Under mitotic asexuality, no ploidy reduction occurs and
136 offspring are clones of their mother.
137 **Meiotic asexuality** (Automixis). Under meiotic asexuality, meiotic divisions occur partially or
138 completely, but somatic ploidy levels are maintained via different mechanisms. Some of
139 these mechanisms have similar genomic consequences as mitotic asexuality, even though
140 meiosis is involved (for example, endoduplication in hybrid asexuals results in offspring that
141 are clones of their mother. Such mechanisms are often referred to as "functionally mitotic"
142 (or functionally apomictic), especially when the cellular mechanisms are not known in detail
143 but genotyping data suggest that offspring are clones of their mother.
144 ***Endoduplication***. A duplication of the entire chromosome set occurs before normal
145 meiosis, during which ploidy is reduced again. If recombination occurs between
146 identical chromosome copies rather than between chromosome homologs,
147 endoduplication produces offspring that are clones of their mother.
148 ***Inverted meiosis with terminal fusion*** (gonoid thelytoky). During the first meiotic
149 division, sister chromatids separate instead of homologous chromosomes. The
150 homologues are separated in the second meiotic division. In the absence of
151 recombination, inverted meiosis with terminal fusion generates offspring that are
152 clones of their mother (and though mechanistically different, is conceptually
153 equivalent to central fusion without recombination). Holocentric chromosomes seem
154 to be a prerequisite for this type of mechanism [43,44].
155 ***Central fusion* and *terminal fusion***. Under these two mechanisms, somatic ploidy
156 levels are restored through the fusion of two of the four meiotic products (products
157 separated during the first meiotic division merge under central fusion, products
158 separated during the second division merge under terminal fusion). In the absence of
159 recombination, central fusion generates offspring that are clones of their mother.
160 ***Gamete duplication***. After a full meiosis, a haploid meiotic product undergoes
161 duplication. This results in a diploid, but fully homozygous offspring.

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

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

202 Mutation accumulation and positive selection

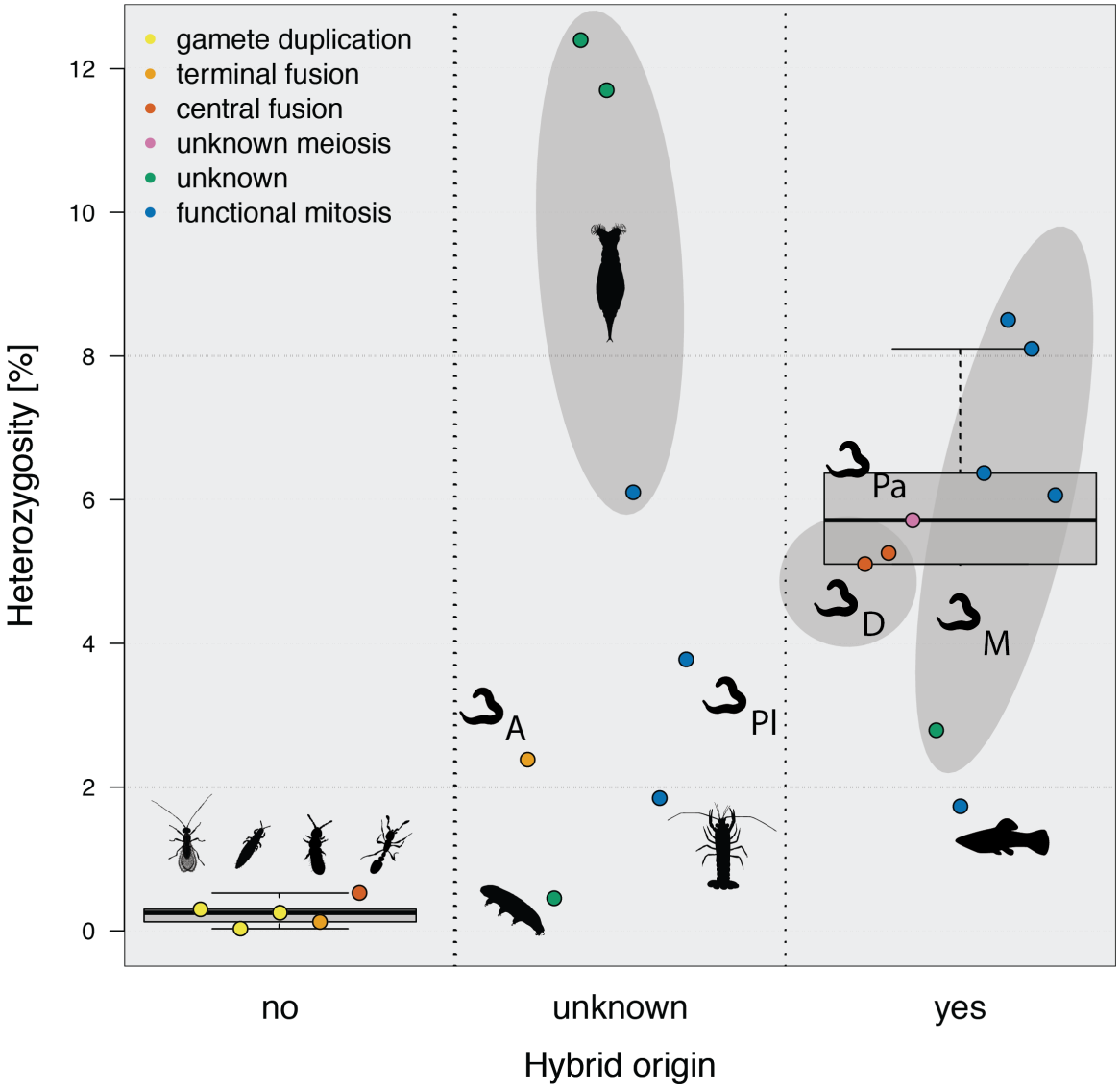
203 One of the classical predictions linked to asexuality is that it reduces the efficacy of selection
204 [5–7,48–50]. This reduction occurs because linkage among loci in asexual species prevents
205 selection from acting individually on each locus. This can allow for deleterious mutations to
206 accumulate over time, because they are linked to other sites under selection. It can also
207 reduce the rate of adaptation, because beneficial mutations cannot reach fixation in a
208 population as easily as under sexual reproduction.
209
210 The prediction that deleterious mutations accumulate more rapidly in asexual than sexual
211 lineages has been tested in over twenty groups of different asexual species (reviewed in [51]
212 and with three additional studies published since [16,21,52]), with results generally supporting
213 the prediction. However, in only eight studies were the tests conducted genome wide, while
214 tests in the remaining studies were based on one or a few genes only. Note that four [11,52–54]
215 of these studies were based on transcriptomes and are therefore not included in our
216 systematic reanalysis. Among the genome wide tests, results are much more mixed than
217 among the 'single or few genes' studies, raising the question whether the latter are
218 representative of the genome as a whole. Specifically, only two of the eight genome-wide
219 studies found support for deleterious mutation accumulation in asexuals [52,53]. However, two
220 studies found that *sexual* taxa experienced more deleterious mutation accumulation than
221 asexual taxa [11,19] while the four remaining ones found no differences between sexual and
222 asexual taxa [16,21,24,54]. In the case of the water flea *D. pulex*, the study specifically reported
223 that earlier inferences of deleterious mutation accumulation under asexuality were incorrect,
224 as the detected deleterious mutations in asexual strains were inherited from the sexual
225 ancestor and did not accumulate after the transition to asexuality [24].
226
227 In summary, results from genome-wide studies addressing the prediction of deleterious
228 mutation accumulation in asexual species are equivocal. More studies are therefore needed.
229 A major constraint for studying deleterious mutation accumulation, and the reason why it
230 was not studied in most genome studies of asexuals species (**Figure 1**), is that it requires
231 sexual references for comparison. Such references are either unknown or not included in
232 most published genome studies of asexuals.

233
234 The same constraints likely explain why no study has thus far addressed adaptive evolution
235 in the genome of an asexual species. The question of adaptive evolution was addressed
236 indirectly in the amazon molly, by studying the amount of segregating variation at immune
237 genes (where variation is known to be beneficial). The authors found very high diversities at
238 immune genes [16]. However, these were difficult to interpret because standing variation was
239 not compared to sexual relatives, and because the amazon molly is a hybrid species. Hence
240 the high diversity could be a consequence of the hybrid origin rather than of asexuality.

241 Heterozygosity

242 Expected heterozygosity levels in asexual organisms are influenced by three major factors:
243 (1) the mechanism of transition to asexuality (which determines the initial level of
244 heterozygosity) **(Box 1)**, (2) the cellular mechanism underlying asexuality (which determines
245 whether heterozygosity should increase or decrease over time) (**Box 2**), and (3) for how long
246 a species has been reproducing asexually (because effects of asexuality accumulate over
247 time).
248
249 As expected, all of the asexual species with a known hybrid origin display high
250 heterozygosity levels (1.73% - 8.5%, **Figure 2**), while the species with an intraspecific origin
251 of asexuality show low heterozygosity levels (0.03% - 0.53%, **Figure 2**). However, it is
252 important to note that hybrid origin is correlated with polyploidy in our dataset, and that
253 heterozygosity does not have a clear definition in polyploids **(Box 3)**. Our measures of
254 heterozygosity are based on the proportion of sites with more than one allele present among
255 all copies, where the total number of copies includes all homologous genome regions **(Box**
256 **3)**.
257
258 The heterozygosity levels present at the inception of asexuality should decay over time for
259 most forms of meiotic asexuality [42,55]. Under mitotic asexuality, heterozygosity is expected to
260 increase over time as haplotypes can accumulate mutations independently of each other
261 (generating the so-called 'Meselson effect')[8]. However, gene conversion can strongly reduce
262 haplotype divergence and, if high enough, can even result in a net loss of heterozygosity
263 over time, even under mitotic asexuality [8,17]. In spite of the prediction that the cellular
264 mechanism of asexuality should affect heterozygosity, the cellular mechanism of asexuality
265 appears to have little or no effect on heterozygosity levels once we control for the effect of
266 hybrid origins (**Figure 2**). However, we have very little power to detect such effects,
267 especially because our dataset does not include any asexual species that uses mitotic
268 asexuality but is not of hybrid origin. Nevertheless, it is interesting to note that species with

269 different forms of meiotic asexuality (including gamete duplication and central fusion) feature
270 similarly low heterozygosity levels. This suggests that although the rate of heterozygosity
271 loss is expected to vary according to mechanisms of asexuality, this variation is only relevant
272 very recently after transitions to asexuality, and no longer affects heterozygosity among
273 established asexual species. Alternatively, variation in heterozygosity caused by different
274 forms of meiotic asexuality may be too small to be picked up with our methods.



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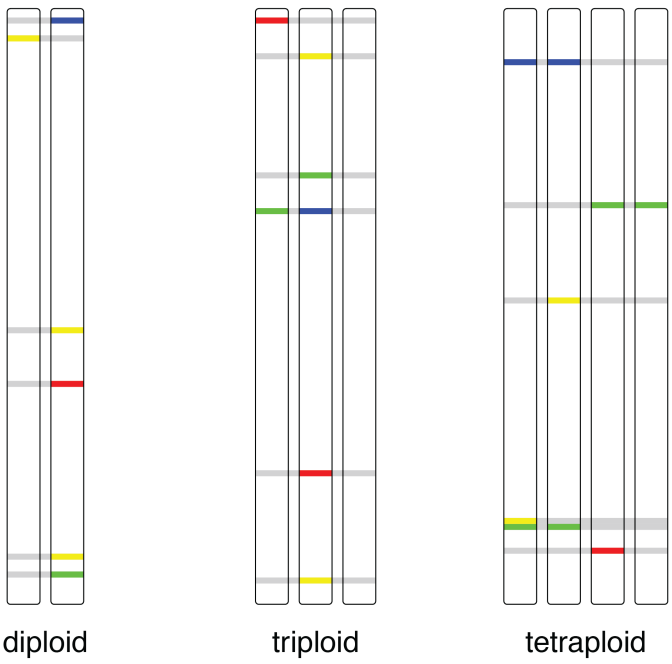
276 **Figure 2**: **Hybrid origin is the main driver of high heterozygosity in asexual species**.
277 Heterozygosity estimates with respect to hybrid origin (x axis) and cellular mechanism of
278 asexuality (color code). Species with a possible shared origin of asexuality are grouped in
279 gray ellipses.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

282 tardigrade *H. dujardini* because of extensive contamination in the sequencing reads, in the
283 water flea *Daphnia pulex* samples because of too low coverage, and in the rotifer *A. vaga*
284 because of divergence levels that exceed the range quantifiable with the applied methods
285 (see **Methods** and **Figure 3**). Heterozygosity is significantly higher in the eight asexuals of
286 confirmed hybrid origin relative to the five that are not (Mann–Whitney U test; p-value =
287 0.0009).
288
289 **Box 3: Quantification of heterozygosity for different ploidy levels**
290 The classical definition of heterozygosity describes heterozygosity as a measure of allelic
291 divergence, where alleles are defined through chromosome pairing [56]. This definition poses
292 a problem for genomes where chromosome pairing is not known (e.g., in polyploid
293 genomes), as well as for genomes of mitotic asexuals where chromosome pairing may not
294 occur at all. In these cases, concepts such as divergence between alleles (i.e.,
295 heterozygosity) *vs.* divergence of paralogs, that are clearly distinct in diploids, become
296 blurred. We therefore quantify heterozygosity as the proportion of nucleotides that differ in at
297 least one of the homologous chromosomes. Three examples of genomes with similar
298 heterozygosity but different ploidy levels are shown on the scheme for illustration. Grey bars
299 highlight specific loci, coloured bars represent alternative alleles at a given locus.



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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

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).

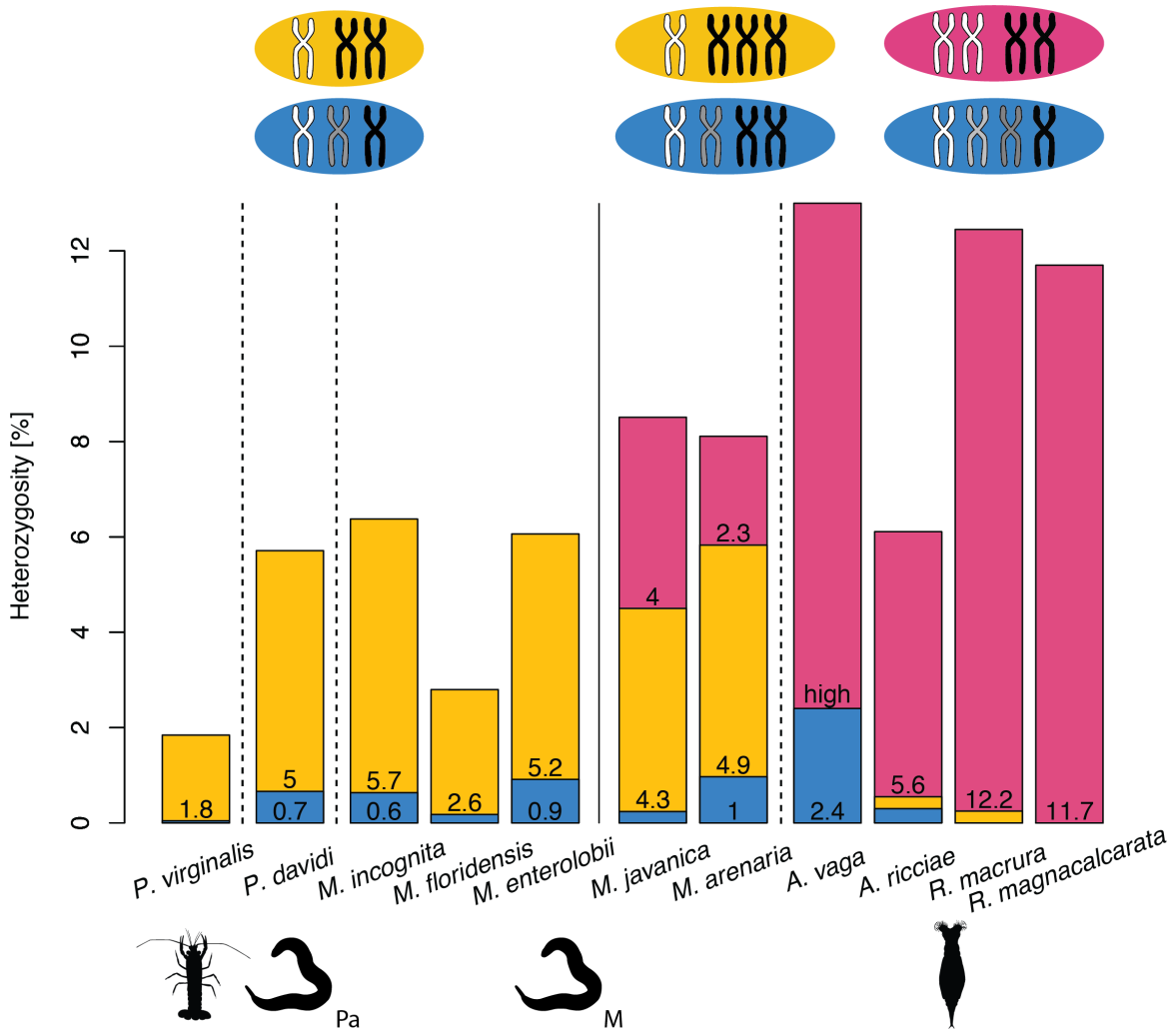


Figure 3: **Heterozygosity structure in polyploids.** Biallelic loci are indicated in yellow or pink:, yellow when the alternative allele is carried by a single haplotype (AAB or AAAB), and pink when both alleles are represented twice (AABB). Loci with more than two alleles are indicated in blue. Note 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

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.

410

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

411

412 * The detected number of palindromes in these species exceeds the number reported in the
413 corresponding genome articles (17 in *A. vaga* and 11 in *F. candida*). This is because we
414 included individual genes in palindromic arrangements, whereas the original genome studies
415 only included genes if they were in palindromic synteny blocks of at least five genes. See
416 also **Supplementary Text S2**.
417

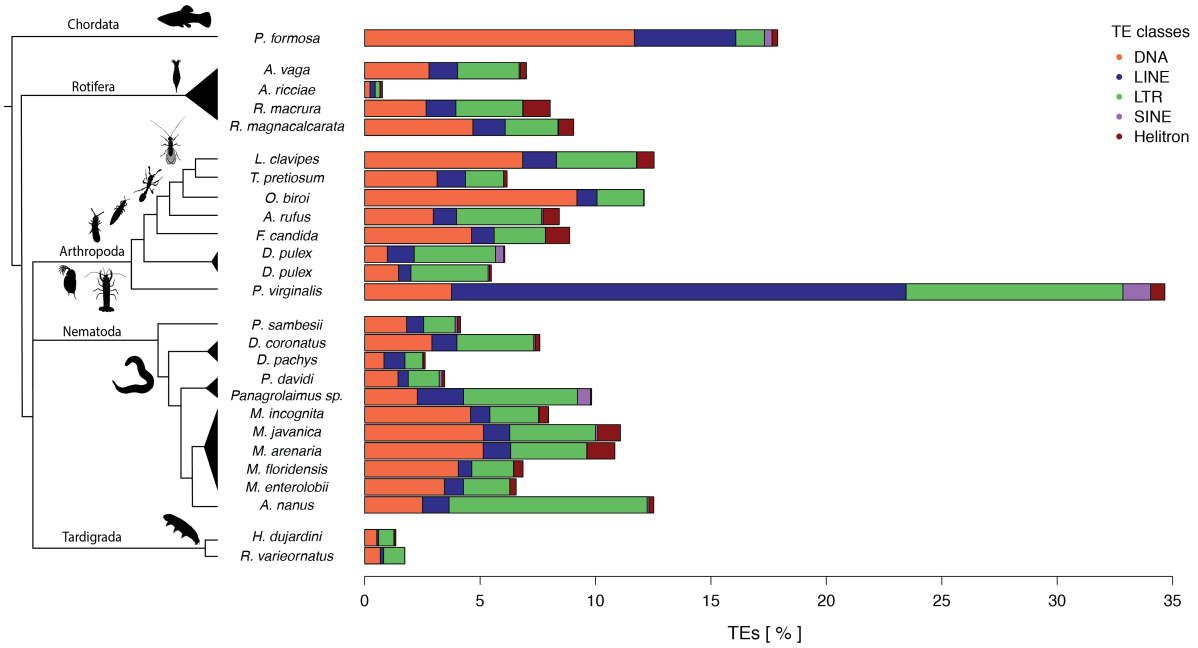
418 Mitotic gene conversion can also occur outside of palindromic regions, for example when
419 double-stranded DNA breaks are repaired using the homologous chromosome as a template
420 [65,66]. It can, in theory, contribute to the loss of heterozygosity under all forms of asexuality,
421 but mitotic gene conversion rates have only rarely been studied in asexual species – or
422 sexual ones for that matter. Gene conversion rates are estimated differently in different
423 studies and are therefore difficult to compare: in the water flea *D. pulex*, they were estimated
424 to amount to approximately 10^{-6} locus^{-1} generation^{-1} [24,25,67], in the amazon molly *P. formosa*
425 to 10^{-8} [16]. Up to 11% of the genome of the nematode *D. pachys* [29] is suggested to be
426 homozygous as a consequence of gene conversion, and studies also argued for an
427 important role of gene conversion for genome evolution in root knot nematodes [34] and
428 rotifers [17,18], although no quantitative estimates are available for these species groups.

429 Transposable elements

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

433 carrying them [70–72]. To the contrary, new TE insertions in coding or regulatory sequences
434 disrupt gene functions and cause deleterious effects in the host; only very rarely can specific
435 insertions be co-opted to acquire novel, adaptive, functions for the host [72]. In sexual
436 organisms, TEs can spread through panmictic populations because of their ability to rapidly
437 colonize new genomes [10,73]. At the same time, sexual reproduction facilitates the purging of
438 deleterious TE insertions, because recombination, segregation and genetic exchange
439 among individuals improve the efficacy of selection [74,75]. In the absence of sex, TEs could
440 therefore accumulate indefinitely, which led to the prediction that TEs could frequently drive
441 the extinction of asexual lineages. Only asexual lineages without active TEs, or with efficient
442 TE suppression mechanisms, would be able to persist over evolutionary times [75]. Consistent
443 with this view, a study in bdelloid rotifers reported extremely low TE loads [76]. This prompted
444 the authors to suggest that bdelloid rotifers could have been able to persist in the absence of
445 sex for over 40 million years thanks to their largely TE-free genomes.
446
447 Our analysis of asexual animal genomes does not support the view that bdelloid rotifers
448 have unusually low TE contents. The TE content of bdelloid rotifers (0.8% to 9.1%) is
449 comparable to other asexual animal taxa (**Figure 4**), all of which are considerably younger
450 than the bdelloids. Across the 24 genomes, there was large variation in total TE content,
451 overall ranging from 6.6% to 17.9%, but with one species, the marbled crayfish, reaching
452 34.7%. Nevertheless, the abundance of TEs in asexual animal genomes appears to be
453 generally lower than in sexual species, which range typically from 8.5-37.6% (median:
454 24.3%) [77]. Whether this difference is indeed driven by asexuality remains an open question
455 as TE loads are known to be highly lineage-specific [20,78]. Furthermore, we annotated TEs in
456 each genome via homology searches in general databases (see methods). This can result in
457 an underestimation of TE loads relative to annotations based on species-specific TE
458 libraries. However, this is unlikely to be of major concern in our study since the methods we
459 used allowed us to identify more TEs than most of the individual genome studies
460 (**Supplementary Table 3**). Specifically, most studies estimate TE loads from genome
461 assemblies, which underestimates TE loads because regions with high repetitive contents
462 are generally not assembled.
463
464 In addition to other lineage-specific characteristics, the cellular mechanisms underlying
465 asexuality could also affect TE loads. For example, most forms of meiotic asexuality can
466 allow for the purging of heterozygous TE-insertions, given the loss of heterozygosity
467 between generations **(Box 2)**. Barring potential gene conversion events, this form of purging
468 cannot occur under mitotic asexuality. However, in the genomes analyzed here, we did not
469 find any effect of cellular mechanisms on TE loads (**Supplementary Figure 3**), likely

470 because the expected effect of the cellular mechanisms is very small relative to lineage-
471 specific mechanisms. Moreover, host TE suppression mechanisms can contribute to the
472 inactivation and subsequent degeneration of TE copies over time, independently of the
473 cellular mechanism of asexuality [72,79].
474
475 Two asexual animals clearly stand out (**Figure 4**), one for very low TE contents (the rotifer *A.*
476 *ricciae;* <1% of the genome) and one for very high contents (the marbled crayfish *P.*
477 *virginalis* >34%). There is currently no known mechanism that could help explain why *A.*
478 *ricciae* differs so extensively from other bdelloid rotifers. In the case of the marbled crayfish,
479 it is unknown whether its extreme repetitive content is a heritage from its sexual ancestor or
480 a consequence of a possible hybrid origin with a subsequent burst of TE activity. In the
481 absence of information on TE loads in the sexual relative *P. fallax,* these possibilities cannot
482 be evaluated. More generally, in most studies quantifying TE contents in asexual species, no
483 comparisons to related sexual species are made. In the cases where this was done, no
484 differences could be detected [16,20,21,26,35,80].
485
486 Independently of the question of whether asexuality affects genome-level TE loads, our
487 dataset allows us to study whether hybrid species have higher TE loads than non-hybrid
488 species. Indeed, TE activity in hybrids is expected to be high because of mismatches
489 between species-specific TEs and silencing machineries [13,15,81,82]. However, we do not find
490 any difference in TE content according to hybrid vs intraspecific origin of asexuals
491 (**Supplementary Figure 2**).



492
493 **Figure 4:** Percentage of transposable elements (TEs) in asexual genomes. Both the TE load

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.

530 ## Horizontal gene transfer

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

552 ## Gene family expansions

553 Most genome papers, including those focussing on asexual animals, scan for expansions of
554 specific gene families. Such expansions are then discussed in the light of the focal species'
555 biology. The expansion of specific gene families *per se* is thus generally a species-specific
556 trait [91] that is not related to asexuality. For example, expansions of stress response genes in
557 *M. incognita* [33], *Panagrolaimus* spp. [30], and *R. varieornatus* [38] were suggested to be
558 associated with the evolution of cryptobiosis in these species. To our knowledge, the only
559 example of a gene family expansion that could be directly associated with asexuality is the
560 diversification of the RNA silencing machinery of TEs in bdelloid rotifers [17]. TEs are expected
561 to evolve reduced activity rates in asexual hosts (see section **Transposable elements**), and
562 an improved RNA silencing machinery could be the mechanism underlying such reduced
563 activity rates.
564

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.

600

601 We have identified hybrid origin as the major factor affecting heterozygosity levels across all
602 asexual animal species with available genomic data. This is consistent with the conclusions
603 of two studies that focussed on individual asexual lineages: hybridization between diverse
604 strains explains heterozygosity in *Meloidogyne* root knot nematodes and *Lineus* ribbon
605 worms [34,54]. This rule applies more generally to all the species analysed with known
606 transitions to asexuality, but it is important to highlight that all the non-hybrid species in our
607 dataset are hexapods. Thus in principle the low heterozygosity could be a hexapod specific
608 pattern, for example due to high gene conversion rates in hexapods. The taxonomic range of
609 the sequenced species is wide but we are missing several clades rich in asexual species,
610 such as mites or annelids [95,96]. These clades would be useful foci for future genomic studies
611 of asexual species. Independently of the findings of such future studies, our results suggest
612 that mitotic gene conversion (that acts independently of palindromes) plays a significant and
613 highly underappreciated role in the evolution of asexual species of intraspecific origin. For
614 example, it has been argued that one of the main benefits of sex could be the masking of
615 recessive deleterious mutations (referred to as “complementation”) which would be exposed
616 under many forms of meiotic asexuality [97,98]. If gene conversion is indeed pervasive, these
617 arguments would extend to functionally mitotic forms of asexuality. Conversely, high rates of
618 gene conversion could also allow for the purging of deleterious mutations while in the
619 heterozygous state, as in highly selfing species (eg. [99,100]). Such purging could help explain
620 why most of the genome scale studies did not find support for the theoretical expectation
621 that asexual reproduction should result in increased rates of deleterious mutation
622 accumulation (see section **Mutation accumulation and positive selection)**. More
623 generally, given the major differences in genome evolution for asexuals of intra-specific vs.
624 hybrid origin, our study calls for future theoretical approaches on the maintenance of sex that
625 explicitly consider the loss *vs*. the maintenance of heterozygosity in asexuals.
626

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

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

genome regions. The sum of coverages of the kmer pairs is then compared against their coverage ratio. This comparison separates different haplotype structures (**Supplementary Figure 1b**). The most prevalent structure is then indicative of the overall ploidy of the genome. We used this ploidy estimate in all species, except *A. vaga*. The most prevalent structure suggested that this species is diploid. *A. vaga* is well characterized as tetraploid [58], but we were unable to detect tetraploidy because homoeologs are too diverged to be identified as such by the kmer-based smudgeplot method.

Using the inferred ploidy levels, we then estimated genome size and heterozygosity using an extended version of GenomeScope [104]. GenomeScope estimates genome wide heterozygosity via kmer spectra analysis, that is, by fitting a mixture model of evenly spaced negative binomial distributions, where the number of fitted distributions is decided given the input ploidy. Estimated distributions correspond to kmers that occur once, twice, etc., in the genome. Fits are then used to estimate heterozygosity, the fraction of repeats in the genome, as well as the 1n sequencing coverage. The latter is subsequently used for estimation of genome size. The definition of heterozygosity for polyploids is not well established (see **Box 3**), but GenomeScope distinguishes different types of heterozygous loci in polyploids (as shown in **Figure 3**).

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

We quantified transposable elements using DnaPipeTE v1.2 [105]. The method uses haploid genome size (parameter -genome_size) to subsample sequencing reads to 0.5x coverage (parameter -genome_coverage). Subsampled reads are then assembled using an assembler that can deal with uneven coverages, and annotated using the database of known TEs. This process is repeated three times (parameter -sample_number), and the union of results represents the repeat library. Additionally, repeats are annotated as TEs if their sequence matches known TEs by homology (for details see [105]). Our reported values of TE loads include only repeats that were annotated as TEs, i.e., we did not include ‘unknown’ repeats which consist of tandem repeats (satellite repeats), duplications or very divergent/unknown TEs.

The palindrome analysis was based on genome assemblies and their published annotations, from 27 samples of 22 species (annotations were not available for *D. pulex* and *A. rufus*).

709 We performed collinearity analysis using MCScanX (untagged version released 28.3.2013)
710 [106], allowing even a single gene to form a collinear bloc (parameter -s) if there were fewer
711 than 100 genes in between (parameter -m). The output was then filtered to contain only
712 blocs on the same scaffold in a reverse order. Furthermore we filtered all the homologous
713 gene pairs that have appeared on the same strand. All the remaining blocks are
714 palindromes, blocs built of reverse complementary genes on the same scaffold. See
715 **Supplementary Text S2** for more details.

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974 **Author contributions:** K.S.J. and T.S. conceived the study, K.S.J. and J.B. collected the
975 data; K.S.J. and T.R.R.B. performed the analyses; K.S.J., J.B. and T.S. reviewed the
976 literature; K.S.J., T.S., J.B. and M.R.R. wrote the manuscript; All authors were involved in
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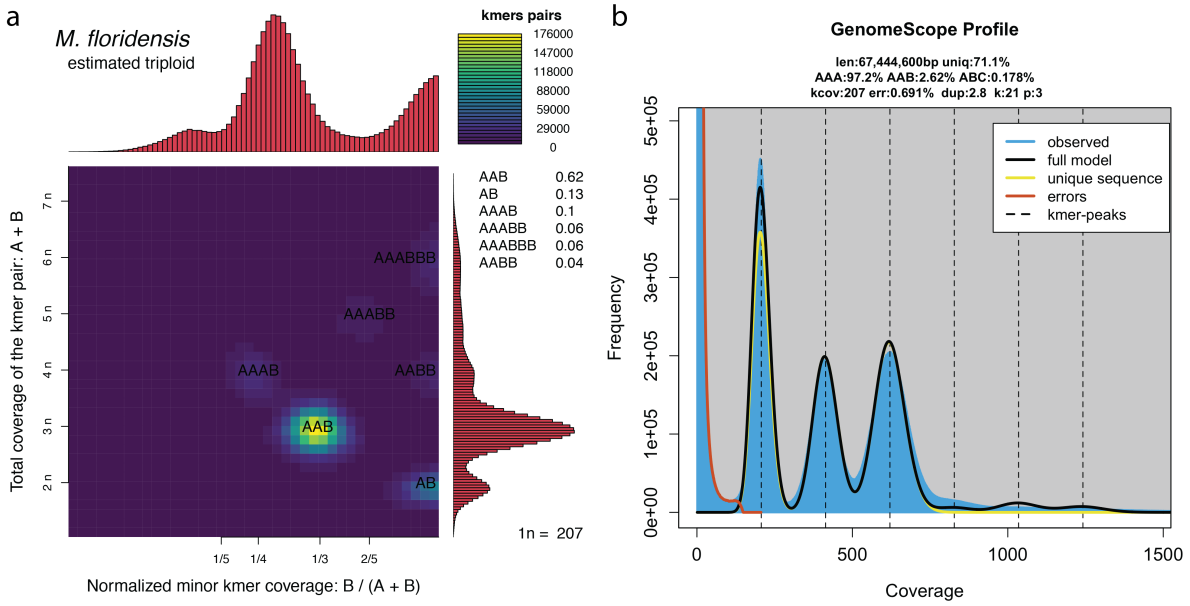
979 Supplementary materials

980 S1 Ploidy and reproductive mode of *M. floridensis*

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

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

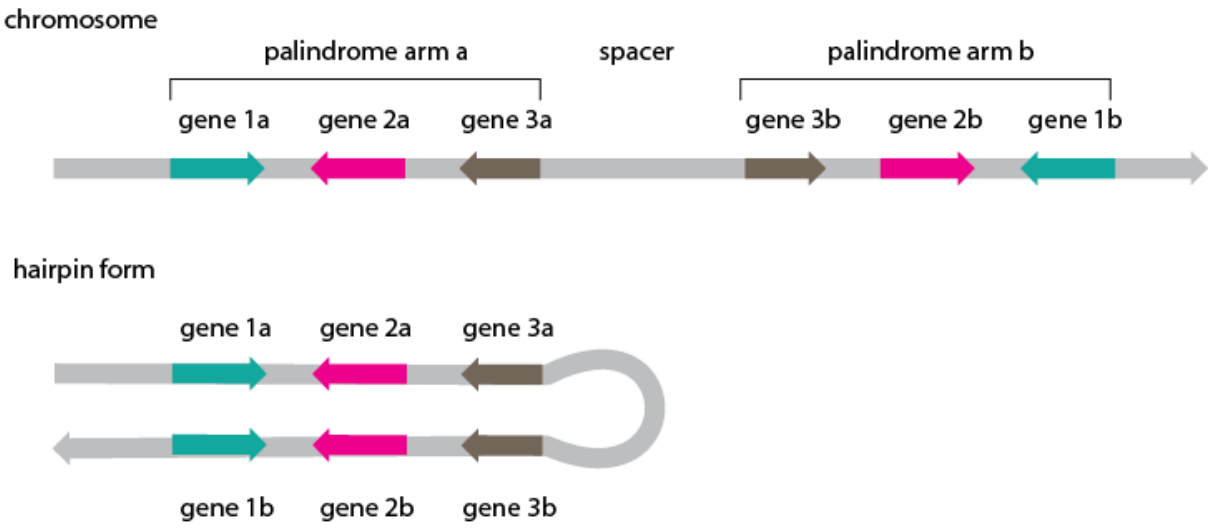


Supplementary Figure 1: Genomic evidence of triploidy in *M. floridensis*. a | the smudgeplot shows dominance of a triploid (AAB) genome structure. The smudges corresponding to higher ploïdies are likely originating from paralogs. The diploid kmer pairs (AB) represent situations where the third allele 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.

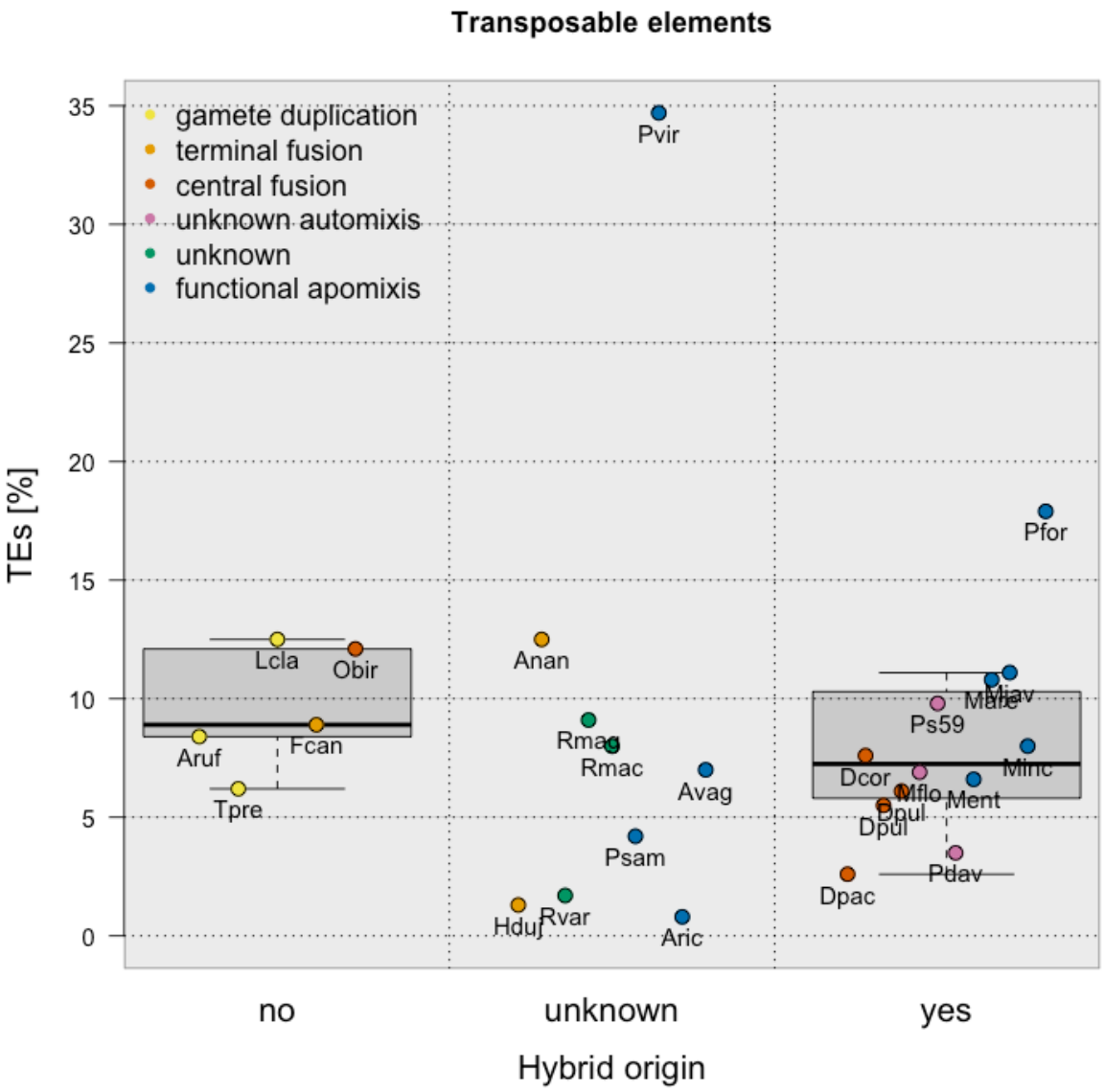
S2 Palindrome detection

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

1016 palindrome could carry fewer than five genes and still be biologically relevant. Detected
1017 collinear blocks were filtered to contain only reverse complementary collinear blocks on the
1018 same chromosome, since only such structures have the capacity to form a hairpin
1019 (**Supplementary Figure 2**).
1020
1021 We note that it is important to check consistency between the biological interpretation of
1022 results, and the methods used to infer them. The bioinformatics pipelines used to detect
1023 palindromes are geared towards detecting large repeated blocks with large gaps. We argue
1024 that small blocks (as small as one gene), but with no gaps within the inverted repeat may
1025 also generate gene conversion. Thus, re-screening the published genomes for palindromes
1026 allowed us to provide a more robust and unbiased view of the importance of palindromes for
1027 the evolution of asexual species. (cit).
1028



1029
1030 **Supplementary Figure 2: Palindrome structure.** The two homologous reverse
1031 complementary regions (arms) of a palindrome are located on the same chromosome. This
1032 organisation allows for the formation of a hairpin and can facilitate gene conversion between
1033 the palindrome arms.
1034

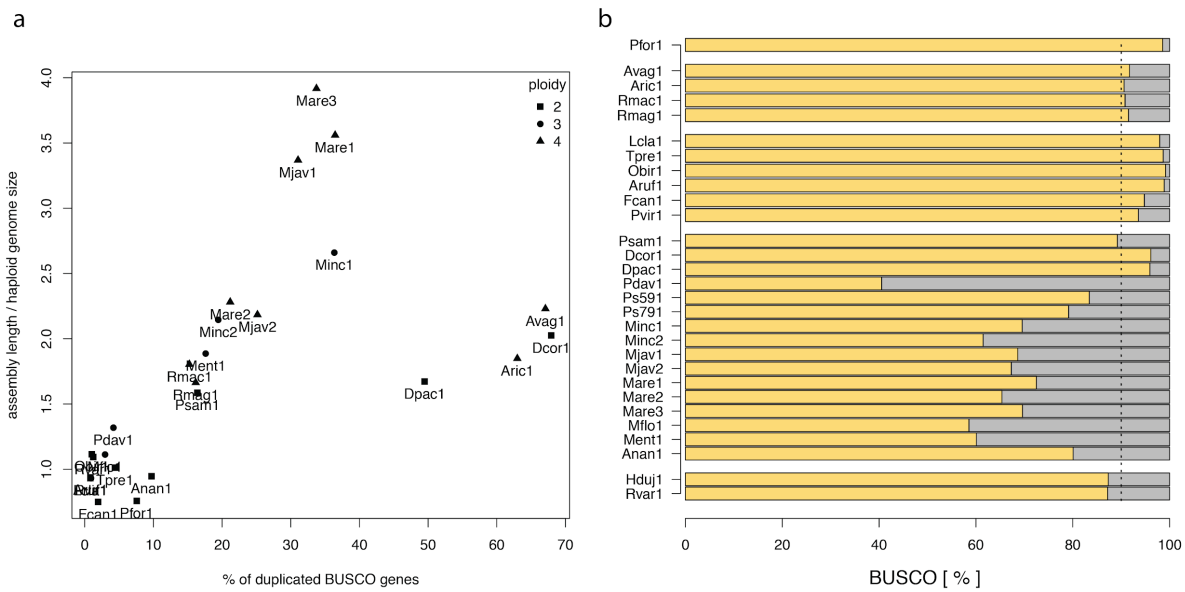


Supplementary Figure 3: Transposable elements with respect to reproduction mode and hybrid origin. Neither hybrid origin (p-value = 0.36) nor cellular mechanism of asexuality (p-value = 0.84) are strong drivers of the TE content in asexual animals.

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

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

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