Within-population genome size variation is mediated by multiple genomic elements that segregate independently during meiosis

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Abstract

Within-species variation in genome size has been documented in many animals and plants. Despite its importance for understanding eukaryotic genome diversity, there is only sparse knowledge about how individual-level processes mediate genome size variation in populations. Here we study a natural population of the rotifer *Brachionus asplanchnoidis* whose members differ up to 1.9-fold in genome size, but were still able to interbreed and produce viable offspring. We show that genome size is highly heritable and can be artificially selected up or down, but not beyond a minimum diploid genome size. Analyses of segregation patterns in haploid males reveal that large genomic elements (several megabases in size) provide the substrate of genome size variation. These elements, and their segregation patterns, explain the generation of new genome size variants, the short-term evolutionary potential of genome size change in populations, and some seemingly paradoxical patterns, like an increase in genome size variation among highly inbred lines. Our study suggests that a conceptual model involving only two variables, (1) a minimum genome size of the population, and (2) a vector containing information on additional elements that may increase genome size in this population (size, number, and meiotic segregation behaviour), can effectively address most scenarios of short-term evolutionary change of genome size in a population.

Introduction

Despite an exponential increase of genomic information during the last two decades, there is still no consensus about the ultimate causes of genome size variation in eukaryotes [1-3]. At the core of this controversy is the puzzling genome size variation across eukaryotic taxa, spanning approximately five orders of magnitude. Genome sequencing has revealed that this variation is primarily caused by gene-, chromosome-, or genome duplications, by variation in the length of introns, number of transposons, and the amount of simple repetitive DNA [4-7]. Since most of these sequences make no substantial contribution to the phenotype, at least not through their information content, genome size is only a poor predictor of organismal complexity [1]. On the other hand, ubiquitous correlations of genome size and cell size, or other phenotypic traits such as metabolic- or developmental rates, and body size [8-12], suggest the sheer amount of DNA in a genome can affect the phenotype[13]. Collectively, this might explain why current theories on genome size variation in eukaryotes differ strongly in their emphasis on selection, mutation and drift [2, 3, 13-16].

Theories invoking selection for increased genome size have been criticized for assuming causal links behind the correlations between genome size and phenotypic traits [2]. Indeed, such correlations often involve species that have been separated for long evolutionary timespans and thus differ in many other aspects than genome size. According to the mutational hazard hypothesis, non-coding DNA is never beneficial, but it may accumulate as a consequence of genetic drift [17]. Thus, this hypothesis offers an alternative, neutral explanation to the observed genome size - phenotype correlations by stating that the accumulation of non-coding DNA in organisms with large body size might be due to their smaller effective population sizes [17, 18]. Testing whether large genome size can sometimes be beneficial, or whether it is at least conditionally deleterious, ideally requires a model system that exhibits substantial genome size differences across a relatively homogeneous genomic background. This requirement appears to be best fulfilled in species with intraspecific genome size variation, where individuals share their genomic background and evolutionary history.

Cases of intraspecific genome size variation are well-documented in plants [19], with cultivated maize and its close relatives being probably one of the best-studied examples [20, 21]. In animals, intraspecific genome size variation has been found in snapping shrimp [22] and in grasshoppers [23]. Interestingly, two intensively studied model species with comparably small genomes, *Arabidopsis thaliana* and *Drosophila melanogaster,* have also turned out to exhibit substantial levels of intraspecific genome size variation [24, 25], suggesting that this phenomenon might be more widespread than previously assumed. Intraspecific genome size variation is sometimes associated with variation in chromosome numbers, for instance due to supernumerary

(B-)chromosomes [19], but there are also documented cases where genome size variation is not reflected the karyotype [22, 26].

Despite its importance, surprisingly little is known about the basic mechanisms and inheritance of intraspecific genome size variation, and the links to population-level phenomena, i.e.: What characterizes those parts of a genome that account for the differences in genome size between individuals? How is genome size inherited by offspring? How (fast) can the trait 'genome size' change through generations, e.g., if it is directionally selected? Even in the best-studied systems, researchers typically rely on assumptions and draw analogies to models of quantitative genetic variation. For example, the trait 'genome size' is often considered a quantitative trait influenced by a large number 'loci', with 'alleles' that increase or decrease genome size [27, 28]. We are not aware of any empirical study that has yet assessed the appropriateness of such a model.

Here we study the basic mechanisms of genome size variation in a population of the rotifer *Brachionus asplanchnoidis*. This species is characterized by an almost two times larger genome size relative to its sister species, by a high 44% genomic content of repetitive elements [29], and by intraspecific genome size variation [30, 31]. Using crossbreeding experiments, selfed lines, and artificial selection, we disentangle the basic mechanism by which variation for genome size is mediated in this population, and how it is inherited by offspring. We capitalize on several advantages of our model system, such as short generation times, sexual and asexual reproduction, and a haploid-diploid lifecycle, which allows us to probe into meiotic patterns associated with intraspecific genome size variation. We could identify the size and number of individual elements that contribute to increases in genome size, and thus account for the gradual differences among individuals in the population. Our findings suggest that intraspecific genome size variation can be conceptualized in terms of a 'minimum genome size' (representing the smallest genomes in a population), and additional 'elements' found in individuals with larger genomes (each characterized by size, number, and meiotic segregation behaviour). This difference in perspective, compared to a conventional quantitative trait-model, has significant implications on the genome size distribution of populations, and the short-term evolutionary potential of the trait 'genome size'.

96 # Results

97

98 **Within-population genome size variation in *B. asplanchnoidis***

99 To quantify genome size variation within populations, we examined 118 *B. asplanchnoidis* clones
100 sampled from four geographic populations (Fig. 1, Supplementary table 1). Of these, two Austrian
101 populations from 'Obere Halbjochlacke' (OHJ, 74 clones) and 'Runde Lacke' (RL, 29 clones) show
102 highly significant intrapopulation variation (OHJ: ANOVA $F_{52,194} = 112$, $P < 0.001$; RL: $F_{28,84} = 23.14$, $P <$
103 0.001). The OHJ-population spans a genome size range of 1.33-fold, from 414 to 552 Mbp (Fig. 1a),
104 with one outlier at 792 Mbp (i.e., 1.91-fold). The RL-population is also variable (Fig. 1b), spanning a
105 range of 1.24-fold across all sampled clones. In contrast, the Lake Nakuru population (11 clones
106 sampled) was not significantly variable ($F_{10,21} = 8.64$, $P = 0.078$), and most genomes were close to 420
107 Mbp (Fig 1c). Previously, we reported two conspecific clones isolated from a Mongolian lake, which
108 have relatively large genome sizes of 652 and 732 Mbp (Fig. 1c, data from Riss et al. 2016). Genome
109 size is mitotically stable, since the genome sizes of clones, as well as the differences among clones,
110 were highly reproducible over a period of more than five years (i.e., approximately 600 asexual
111 generations, Supplementary Fig. 1).

112

113 **Inheritance of within-population genome size variation**

114 Clones with divergent genome size can mate with each other and produce viable and fertile
115 offspring, which are intermediate in genome size between their parents but show some variation
116 (Supplementary Fig. 4, see also [32]). Genome size responds to artificial selection with extremely high
117 heritability. We applied truncation selection to the OHJ population by crossing clones with the 10%
118 largest genome sizes among each other (excluding the outlier clone at 792Mbp), and by crossing
119 clones with the 10% smallest genomes, respectively. In the up-selection treatment, we obtained
120 genome sizes exceeding the range of the parental OHJ population (Fig. 2). We could select genome
121 sizes of up to 640 Mbp, with a narrow-sense heritability h^2 of 0.905 in the first generation, and 0.912
122 in the second generation. Likewise, heritability was high in the first generation of the down-selection
123 treatment ($h^2 = 0.924$). However, in contrast to selection for large genome size, it did not extend the
124 range of the original population. In fact, we could not select genome sizes below 414 Mbp.
125 Additionally, h^2 of the second generation of the down-selection treatment collapsed to zero. A
126 parent-offspring regression including all our crosses yields an overall estimate for h^2 of 0.96
127 (Supplementary Fig. 5).

128 Combining all available genome size estimates of *B. asplanchnoidis* reveals a positively
129 skewed distribution, with a high number of observations at 410-430 Mbp and an elongated tail of
130 large genome sizes (Supplementary Fig. 6). The striking absence of genome sizes smaller than 410
131 Mbp, despite high sampling effort and intentional selection for small genome size, suggests that this
132 genome size might be a true biological limit. By contrast, there was no constraint in terms of
133 increases in genome size, as suggested by the more or less continuous rise of genome sizes to 792
134 Mbp. Interestingly, this clone with the largest genome size was not artificially selected, but hatched
135 from a resting egg of the natural population (c.f., Fig. 1a).

136 To get additional insights into the inheritance of genome size, we analysed two selfed lines
137 that were derived from a clone with large and small genome size, respectively. Theoretically, one
138 would expect trait variation to decrease upon selfing as this causes 50% reduction of heterozygosity
139 each generation. In contrast, we found that genome size variation remained high in a selfed line that
140 descended from a large-sized clone (524 Mbp). Genome sizes of selfed offspring ranged between
141 values of 522 Mbp and 644 Mbp, representing a 23% increase (Fig. 3). Among-clone variation in the
142 large selfed line was statistically significant, even after three generations of selfing (ANOVA $F_{9,24}$ =
143 53.61, $P < 0.001$). Variation in the large line was also significantly higher than in the line descending
144 from the small genome ($p < 0.001$; R package *cvequality*, version 0.2.0, [33]). After three generations
145 of selfing, we performed a sexual cross between both lines. As was the case in the cross between two
146 natural clones, the cross of the selfed lines yielded offspring that were variable and intermediate
147 between their parents (Fig. 3).

148

149 **Evidence for independently segregating genomic elements**

150 To gain mechanistic insights into segregation of genome size variation during meiosis, we
151 analysed haploid rotifer males. In a diploid organism with equally sized chromosome pairs and no
152 extra-chromosomal elements, all meiotic products should contain exactly half the DNA of a diploid
153 cell. Consistently, haploid males of the rotifer *B. calyciflorus* have half the genome size of females
154 ([34]; see Appendix S2 in this publication). We also find this pattern in some of our *B. asplanchnoidis*
155 clones (e.g. Fig. 4a). However, in many others we obtained striking variation in male genome size,
156 which manifested in multiple discrete “male peaks” (Fig. 4b-h, Supplementary table 2). In the
157 simplest case, we observed two male peaks spaced symmetrically around the expected 1C-value (Fig.
158 4b). In general, the male peak pattern of a clone could be characterized by (i) the number of peaks,
159 (ii) an odd/even number of peaks, indicating the presence/absence of a central male peak at exactly
160 1C, and (iii) the relative abundance of certain male genome size classes, as inferred from the area

161 under each male peak. In most cases, the central male genome sizes (the ones close to 1C) were
162 more abundant than the ones in the periphery (Supplementary Fig. 7, Supplementary table 2).
163 Overall, male peak patterns were clone-specific and highly repeatable (Supplementary Fig. 8).

164 In some clones, we could not resolve individual male peaks, but instead obtained an
165 extremely broad distribution of genome sizes around the 1C value (e.g., Fig 4f). This pattern likely
166 reflects real genome size variation among males of a clone, rather than a lack of measurement
167 precision, since the coefficient of variation of male genome sizes in this example was 12%, while
168 variation of the female peak ~2%. Interestingly, this clone with the extremely broad male peak was
169 the same as the "outlier" with the largest genome size of 792 Mbp (Fig. 1a). Although this clone was
170 the extreme case, we obtained broad distributions of male genome sizes in other clones as well (data
171 not shown). Finally, in some clones we observed characteristically unequal distances between male
172 peaks (Fig. 4 g,h), such that some male peaks were more narrow together than others.

173 All the subsequent results and analyses build on the following *ad hoc* hypothesis, which is
174 based on our observations so far: We hypothesized that large genomic elements, several Mbp in size,
175 are segregating independently from each other, and thus give rise to discrete classes of male genome
176 size. By independent, we mean that each element has an approximately equal chance of segregating
177 into any of the four gametes during gametogenesis. Note that this is different from normal
178 chromosome segregation, where homologous chromosomes will always end up in opposite gametes.
179 The presence of one independently segregating element can explain the simplest pattern of two
180 male peaks (Fig 4b), being present in the large male genome size but absent in the small one.
181 Similarly, two equally sized elements can explain a pattern of three male peaks (Fig. 4c), which
182 correspond to genome size classes with zero, one, or two elements. Likewise, patterns with higher
183 numbers can be explained by *n*-1 elements. Assuming that independently segregating elements have
184 identical size, we can predict the relative ratios of the male genome size classes to 1:1 (one element),
185 1:2:1 (two elements), or 1:3:3:1 (three elements). Indeed, some of our clones closely follow these
186 predicted frequencies, whereas others showed some deviations (Supplementary fig. 7). For example,
187 in clones with two male peaks, the smaller male genome size was often at higher frequency than the
188 expected value of 0.5 (Supplementary fig. 7), suggesting that males without an element were more
189 frequent among the hatched males. Likewise, in clones with three and four male peaks, the central
190 male genome sizes were sometimes at a higher frequency than expected. One clone showing four
191 male peaks with almost identical heights (Supplementary fig. 7c) presents a particularly interesting
192 deviation. In this same clone, the two central peaks were closer together (cf. Fig. 4g). The most
193 parsimonious explanation seems to be that this clone carries two differentially sized elements, a
194 small and a large one, and that the four male peaks correspond to: (1) zero elements, (2) the small

195 element, (3) the large element, and (4) both elements. Likewise, two large and one small element
196 can explain the "three double-peaks" pattern in the clone depicted in Fig. 4h.

197 With this in mind, we estimated the size and number of independently segregating elements,
198 based on the distance between male peaks in a clone and its 2C genome size (Fig. 5a). We find that
199 the natural OHJ-population harbours a large diversity of elements (Fig. 5b). Many clones contain
200 elements of ~34Mbp size, but smaller elements down to 15 Mbp were also present in this
201 population. In contrast, the clones of our "large" selfed line apparently contained only elements in
202 the 34 Mbp size range (Fig. 5b), and they exhibited significantly less variation in element size than the
203 natural population ($p<0.001$; R package *cvequality*, version 0.2.0, [32]). Interestingly, the founding
204 clone of this line shows four male peaks (Fig. 4d), and indicated three elements of ~34Mbp size. Thus,
205 it appears that this same 34Mbp element causes all the observed genome size variation in the "large"
206 selfed line, being present in different numbers in different offspring.

207

208 **Link between independently segregating elements and genome size variation**

209 The hypothesis of independently segregating elements offers an explanation to many
210 patterns in our data. However, does it provide a *sufficient* explanation for within-population genome
211 size variation? To explore this idea, we formalized our findings in a quantitative model of multiple
212 independently segregating elements (for details and code, see Supplementary Methods). We
213 assumed a minimum diploid genome size at 414 Mbp and that each additional element in a clone
214 would proportionally increase its genome size. Thus, the main input variable of the model is a vector
215 specifying the size and number of elements in a clone. We assumed that all elements segregate
216 completely independently from each other. Thus, if a clone contains, for example, five elements,
217 there is a small chance that it produces males with zero or five elements, while the majority of males
218 will contain two or three elements. In the model, we also defined a parameter for the precision of
219 the flow cytometry measurement, in terms of the coefficient of variance (CV). We set its default
220 value to 2.7%, reflecting the overall mean in our male peak data (Supplementary table 3), but we also
221 explored a CV of 2%, which we obtained in some of our best samples. On the whole, our model can
222 reproduce virtually all male peak patterns found in *B. asplanchnoidis*, and it illustrates some
223 technical issues, e.g., how measurement precision limits the detection of the fine patterns produced
224 by small elements or when a mixture of different element sizes are present in a clone
225 (Supplementary Figs. 9, 10). We could also reproduce the peculiar case of clone OHJ72, our outlier of
226 the natural OHJ-population, with its extremely broad male peak. According to our model, this clone
227 might harbour eleven 34Mbp-elements, or even more, if these elements are smaller.

228 One method to test whether independently segregating elements provide a sufficient
229 explanation to genome size variation is to "predict" genome size of a clone based on the number and
230 size of its elements. Although this prediction is recursive, since it requires knowledge of female
231 genome size for sizing of the elements, it is not circular, because the distance between male peaks is
232 free to vary. Thus, if we should find that predicted and observed genome sizes differ greatly, this
233 would suggest that independently segregating elements are *not* a sufficient explanation for genome
234 size variation. To explore this idea, we analysed the crossed offspring of the two selfed lines (cf., Fig.
235 3). This cross was especially suitable because of their low diversity of segregating elements (Fig. 5b,
236 Supplementary table 2). We obtained striking agreement between predicted and observed genome
237 sizes, and could account for 96% of the diploid genome size, on average, just based on the number of
238 34Mbp elements (Fig. 6a). Nevertheless, all observed genome sizes were approximately 18 Mbp
239 higher than the predicted ones, indicating that there might still be some variation that the model
240 does not account for.

241 Identifying the exact size and number of independently segregating elements is not possible
242 for the natural OHJ population as a whole, due to the higher diversity of segregating elements in this
243 population (Fig. 5b), and due to limited resolution of male peak patterns in clones containing
244 multiple differentially sized elements (Supplementary Fig. 9). To circumvent these limitations, we
245 tested whether the amount of meiotically segregating genome size variation is correlated with
246 genome size. To this end, we defined the variable RCV (Relative Coefficient of Variation), which is the
247 CV of all combined male genome sizes of a clone divided by the CV of the female genome size. Thus,
248 RCV accounts for differences in measurement precision across samples (indicated by the CV of the
249 female peak), while it requires only that a clone can produce males in order to be measured, thus
250 extending our analyses to many more clones (n=72). We found that the RCVs of OHJ-clones were
251 considerably higher than those of outgroup rotifer species without genome size variation (Fig. 6b),
252 and that genome size and RCV were positively correlated (Spearman rank correlation, $\rho = 0.89$, $P <$
253 0.001). Consistently, RCVs in the "large" selfed line were significantly higher than in the "small"
254 selfed line (Fig. 6c; Mann Whitney U-Test, $W = 88$, $P < 0.001$). Our model predicted the same
255 curvilinear relationship between genome size and RCV as we observed in *B. asplanchnoidis*, assuming
256 either 20Mbp or 34 Mbp elements (Supplementary Fig. 11). In particular, the prediction based on 20
257 Mbp elements seemed to match the majority of clones of the OHJ population. Altogether, the
258 positive correlation of RCV with genome size as well as the congruence of the model predictions and
259 the natural OHJ-population suggest that independently segregating elements are largely responsible
260 for genome size variation in *B. asplanchnoidis*.

261

262 # Discussion

263 **Extensive within-population genome size variation in B. asplanchnoidis**

264 Here we report a case of within-species genome size variation in the rotifer *B. asplanchnoidis*
265 and show that even individuals *within* a natural population can differ by 1.33-fold, exhibiting genome
266 sizes ranging from 414 to 552 Mbp. Moreover, artificial selection for large genome size can extend
267 this range to 1.55-fold (640 Mbp) within just two generations. To our knowledge, these ranges
268 represent some of the most pronounced cases of intraspecific variation in animals and plants
269 (Supplementary Table 4).

270 Claims of intraspecific genome size variation have sometimes met scepticism, due to possible
271 methodological artefacts [35], or the species concept underlying the study [36]. Thus, we used two
272 different internal standards in our flow cytometry measurements, either *Drosophila melanogaster*
273 flies or other rotifer clones that were co-prepared with a focal sample, to demonstrate genome size
274 differences among rotifer clones. Both internal standards yield virtually identical results, confirming
275 that our genome size estimates are accurate and reflect real differences among clones
276 (Supplementary Figs. 2, 3). Previous studies have documented the species status of *B. asplanchnoidis*
277 [32, 37], and have shown that the populations from Austria, Mongolia and Lake Nakuru are
278 genetically distinct, but also experience natural gene flow on a larger geographic scale [32]. Thus, in
279 the case of *B. asplanchnoidis* we can exclude any unrecognized cryptic species.

280

281 **Independently segregating elements are responsible for genome size variation**

282 Within-population variation of genome size in *B. asplanchnoidis* is mediated by multiple, large
283 genomic elements that segregate independently during meiosis. These elements range between 15
284 and 34 Mbp in size and are present in variable numbers in individuals of the natural population.
285 Thus, they account for the full range of genome size variation in this population, including the
286 apparent "outlier" at 792 Mbp. Individuals with the smallest observed genome size are free of such
287 elements, while individuals with larger genomes contain progressively more elements, in different
288 numbers and combinations. Altogether, this allows for a seemingly gradual distribution of genome
289 sizes across the whole population.

290 The meiotic behaviour of these elements is unusual in that they segregate independently
291 from each other. This has important implications for maintenance of genome size variation in this
292 population, because it facilitates the (re-)generation of a large number of genome size variants in
293 sexual offspring, even if the two parents have identical genome size. For example, consider two

294 parents with three 20 MBp and two 30 Mbp elements, respectively. Both have the same genome size
295 (414 + 60 = 474MBp), but due to independent meiotic segregation of these elements, and
296 recombination, their crossed offspring can have one of ten different genome sizes and range
297 between 414 and 534 Mbp. There is some resemblance to allelic recombination in a genetically
298 controlled trait determined by multiple loci. However, in the case of genome size, variation can even
299 be generated in genetically highly homogeneous lines, such as selfed lines.

300 On a chromosome level, independently segregating elements in *B. asplanchnoidis* may
301 resemble supernumerary chromosomes (B-chromosomes) that do not pair during meiosis. This is
302 suggested by our results from the selfed line, which seems to have accumulated up to six identical
303 elements derived from an ancestor with three elements. If so, the B-chromosomes are quite large in
304 their relative size, since one 34Mbp element corresponds to 8.2% of the minimum diploid base
305 genome observed in this population. B-chromosomes reported in previous studies were often
306 smaller than normal chromosomes [38], but there are also reports where they reach 3-5% of the
307 diploid genome size [23, 38]. On the other hand, regarding absolute size, B-chomosomes of other
308 species can be twice as large the whole genome size of *B. asplanchnoidis* [23]. A second possible
309 mechanism is that the extra DNA is integrated into the normal set of chromosomes, thus resulting in
310 homeologous chromosomes of different length [e.g., 26]. According to this hypothesis, simple
311 patterns of two or three male peaks could be caused by one or two elements located on different
312 chromosomes, being present only once per chromosome pair. Unfortunately and despite much
313 effort, we could not obtain reliable information on the karyotypes of *B. asplanchnoidis*, which might
314 have allowed us to distinguish between these two possibilities, likely because cell divisions in this
315 organism are limited to a short period when the embryo is encapsulated in a robust eggshell.
316 However, even though we cannot completely rule out the second mechanism, supernumerary
317 chromosomes appear to be the most plausible explanation based on our selfed lines. After all, the
318 two mechanisms are not mutually exclusive. For example in maize, B-chromosomes and
319 "heterochromatic knobs" can both contribute to intraspecific genome size variation [19]. Regardless
320 of whether these independently segregating elements are located on supernumerary chromosomes
321 or integrated in the regular set of chromosomes, we can draw important general conclusions about
322 intrapopulation genome size variation.

323

324 **Towards a general model of within-species genome size variation**

325 The model developed here requires as parameters only a minimum genome size (for individuals free
326 of elements), a vector describing size and number of all elements within a clone, and information

327 about their meiotic segregation patterns. This model is consistent with high heritability of genome
328 size and the strong response to selection, while it accounts for the fact that we could not select
329 below a minimum genome size. It is worth mentioning that the minimum genome size that we
330 defined for our population was not obvious from the distribution of the natural population (Fig. 1a),
331 but could only be established by experimental manipulation. Interestingly, previous reports of
332 intraspecific genome size variation in other organisms also report positively skewed distributions (22,
333 34), which could be an indication of a minimum genome size. Our model could reproduce virtually all
334 observed meiotic segregation patterns ("male peaks"), and it is consistent with the increase of male
335 segregational variation (RCV) with genome size that we observe in the OHJ population (Fig. 6b).
336 Finally, this model can explain the unexpectedly high genome size variation in a selfed line derived
337 from a clone with large genome size.

338 Our model differs in several notable aspects from previous conceptual models on genome
339 size that were adopted from a perspective of quantitative trait (QT) models. Importantly, QT-models
340 are defined in terms of the mean genome size of a population governed by a large number of
341 insertion and deletion alleles (24). According to our model, QT-models thus refer to the special case
342 where all members of the population have an elevated genome size. However, observations like the
343 minimum genome size at ~414MBp in the OHJ population, or the fact that genome size variation
344 remained high in the selfed line, suggest that the QT-model might not capture all aspects of the
345 genome size dynamics. In addition, a focus on minimum genome size and DNA-additions, rather than
346 on deletions, is more consistent with the notion that the largest fraction in eukaryotic genomes is
347 excess junk DNA [39]. Nevertheless, it is important to realize that our concept of a 'minimum genome
348 size' is not equivalent to a minimal possible genome, but that it is operationally defined, and includes
349 all DNA-additions that are invariably "fixed" in a specific population, or species.

350 Our present model may be generalized further. Currently, it is strongly oriented towards a
351 mechanism that involves completely independent segregation of extra DNA, which might be best
352 represented in the case of B-chromosomes. However, it can be extended to include other
353 mechanisms, e.g., where extra genomic material is located on normal chromosomes. Accounting for
354 the latter would require some constraints regarding segregation patterns of extra DNA, particularly if
355 these elements are located on the same chromosome. Other variables and parameters, such as
356 minimum genome size and size of elements, would be largely unaffected. Such a generalization has
357 interesting consequences. For example, extra genomic material may become 'fixed' in a population
358 once all members carry the same extra DNA element on both chromosomes. Ultimately, a general
359 model would incorporate both mechanisms, integration to normal chromosomes and supernumerary
360 chromosomes, and perhaps even an exchange between both pools [40-43]. Thus, genome size could

361 be highly dynamic on short time scales, due to mechanisms that involve independent segregation,
362 while fixation/loss of extra genomic material on the normal set of chromosomes could explain the
363 long-term changes in genome size across populations or species. Overall, this model does not even
364 contradict long-term gradual (Brownian motion) changes in genome size, while it adds a population-
365 level perspective to taxa that seem to have undergone "saltations" in genome size over
366 macroevolutionary time scales (e.g., [44])

367

368 ## Conclusions

369 We have shown that within-population genome size variation in the rotifer *B. asplanchnoidis*
370 is mediated by relatively large genomic elements that segregate independently of each other during
371 meiosis. Our data on short-term artificial selection and inbred line variation suggest that a model that
372 involves only two variables, a minimum genome size and a vector specifying the number, size, and
373 segregation behaviour of the elements that increase genome size is sufficient for capturing most
374 aspects of genome size dynamics in this population. Collectively, our study closes an important gap in
375 our knowledge of how intraspecific genome size variation in populations is mediated by processes at
376 the individual level. Since the genome size variations in this model system are realized across a
377 relatively homogeneous genomic background, this has general implications for identifying the
378 evolutionary forces that are responsible for the immense variation of genome sizes seen across
379 eukaryotes. Most notably, future studies in this system should allow disentangling whether larger
380 genome size can be beneficial, or whether it is always slightly deleterious. In the future, it will also be
381 interesting to elucidate in more detail the mechanisms behind these independently segregating
382 elements, for example, their underlying genomic architecture, or how they interact with the "non-
383 dispensable" parts of the genome.

384

385 ## Methods

386 Resting eggs of rotifers were collected in the field from Obere Halbjochlacke (OHJ, N 47°47‘11“,E
387 16°50‘31“) and from Runde Lacke (N 47°47‘08“, E 16°47‘34“), two small alkaline playa lakes in
388 Burgenland (Austria) in 2011. Resting eggs from Lake Nakuru (Kenya) and the two Mongolian clones
389 were obtained from colleagues and have been previously described in detail [30, 32]. All rotifers
390 were cultured as clones, consisting of the asexual descendants of the female that initially hatched
391 from a resting egg (for details, see Supplementary methods).

392 Genome size measurements were performed with flow cytometry using a detergent-trypsin
393 method and propidium iodide (PI) staining according to [30] with minor modifications (for details,
394 see Supplementary methods). As an internal standard of known genome size, we used the fruit fly,
395 *Drosophila melanogaster* (strain ISO-1, diploid nuclear DNA content: 0.35 pg; [45]).

396 For sexual crosses between two rotifer clones, we used freshly hatched virgin females and
397 males, which were harvested as eggs from dense rotifer cultures that had initiated sexual
398 reproduction (for details, see Supplementary methods). To analyse inheritance of genome size within
399 a population, we crossed two clones with divergent diploid genome size (414 versus 524 Mbp; called
400 ohj22 and ohj7, respectively) and analysed 27 of their sexual offspring. Two selfed lines were
401 established from the same two clones by growing mass cultures until they produced resting eggs
402 (which were the product of self-fertilization, i.e., males fertilizing females of the same clone). Selfed
403 lines were propagated for three sexual generations by randomly selecting one offspring clone each
404 generation. Finally, we randomly selected one offspring clone from the “large” and “small” line and
405 cross-mated them to produce an inter-line cross.

406 For the artificial selection experiments, we applied truncation selection to the natural OHJ
407 population by crossing eight clones representing the 10% largest genome sizes among each other
408 (excluding the outlier clone at 792Mbp), and by crossing eight clones representing the 10% smallest
409 genomes, respectively. In the first generation, we used 14 combinations of parental clones for each
410 selection treatment and analysed 1-9 of their offspring. In total, the F1-generation encompassed 31
411 and 26 offspring clones for the large and small selection-line, respectively. We repeated this selection
412 procedure in the F1-generation to produce a F2-generation. In the small selection line, we used five
413 clone combinations to produce 15 F2-offspring clones. In the large selection line, sexual propensity of
414 the F1-clones was extremely low, limiting us to just one clone combination and 14 of their offspring
415 in F2. The exact genealogy of clones in the F1- and F2-generation can be inferred from their names
416 listed in supplementary table 1. Narrow sense heritabilities h^2 were estimated using to the “Breeder’s
417 equation” $\Delta Z = h^2 \cdot S$, where S is the selection differential, and ΔZ is the response to selection.
418 Additionally, we calculated h^2 from the slope of the best-fit line for a plot of mid-offspring versus

419 mid-parent genome size of all our available data, which included all crosses and the self-fertilized
420 clones.

421 To determine the genome size of males relative to (diploid) females, we grew clones from
422 low to high population densities until they started to produce males. We co-prepared males and
423 females from the same clone and subjected them to the same protocol as above, except that we did
424 not use a *Drosophila* internal standard. To better visualize male peaks, we used an excess of males,
425 from 200 males + 100 females to 300 males + 60 females, depending on genome size of the clone. In
426 the flow-cytometry analysis, we quantified the following variables: number of male peaks (up to 6),
427 position of each individual male peak (as the median of the YL-2A value), position of the female peak,
428 standard deviation (YL-2A value) of all combined male peaks, and standard deviation of the female
429 peak. In contrast to the previous genome size measurements, we applied a slightly stricter precision
430 cut-off at 3.5% CV of the diploid female peak, and discarded all samples with higher CVs.

431 We performed two types of analyses depending on the quality our male peak data. First, in
432 clones that showed multiple discrete male peaks, we counted and sized these peaks (relative to the
433 female peak). We also determined the area under each peak, as a measure for the frequency of each
434 male genome size class. Second, for these and for all remaining clones, we calculated relative
435 coefficient of variation (RCV) according to

436 $$RCV = CV_{allMP}/CV_{FP}$$

437 , where CV_{allMP} is the CV across all male peaks and CV_{FP} is the CV of the female peak. Thus, RCV is a
438 measure for male genome size variation within a sample, corrected for its measurement error
439 (indicated by the CV of the female peak). To obtain a point of reference for RCV values in species
440 without intraspecific genome size variation, we conducted the same analyses in four sister species (*B.*
441 *rotundiformis*, *B. plicatilis*, *B. manjavacas*, B. 'Nevada').

Figures and figure legends

Fig. 1

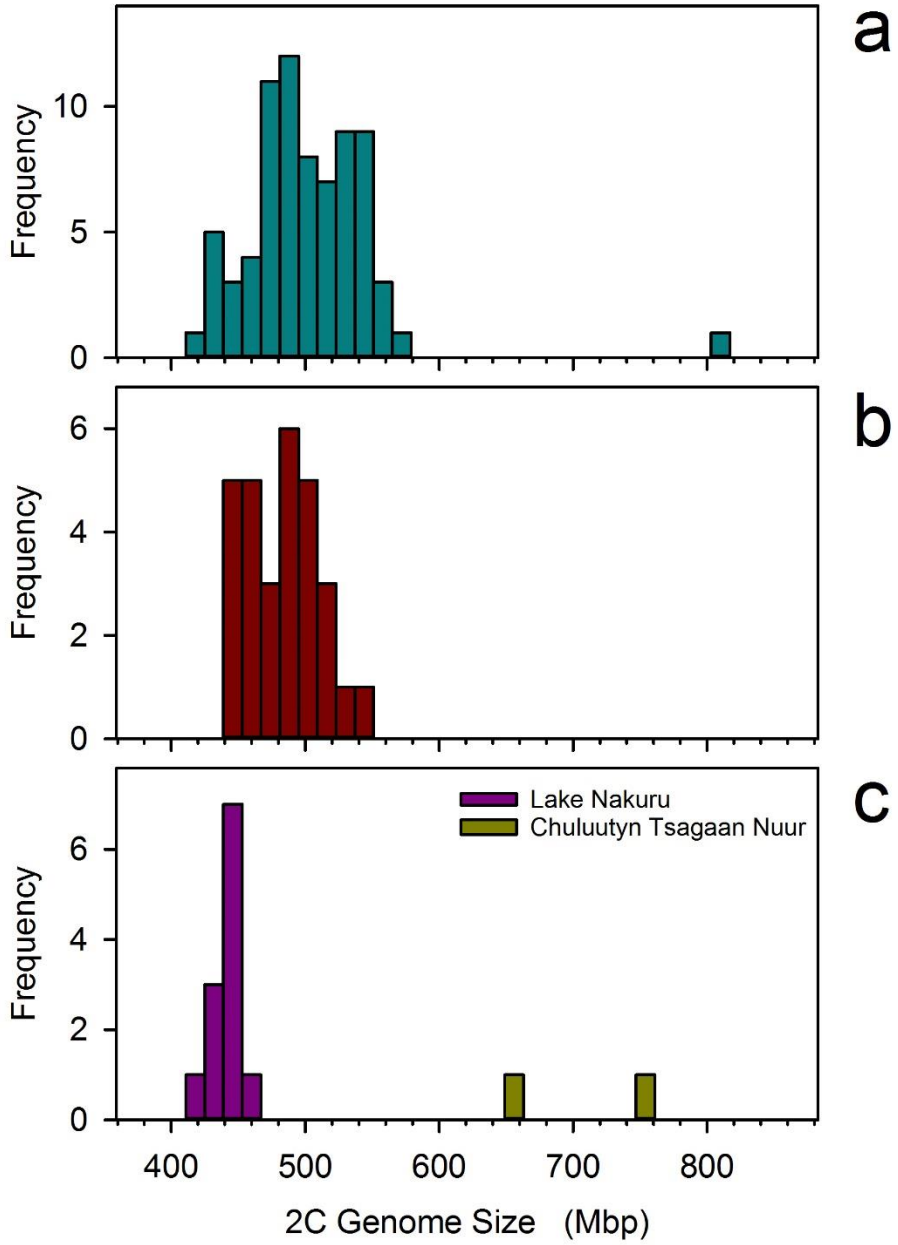

Fig. 1 Genome size variation in four natural *B. asplanchnoidis* populations. **a** Obere Halbjochlacke (Austria). **b** Runde Lacke (Austria). **c** Lake Nakuru (Kenya) and Chuluutyn Tsagaan Nuur (Mongolia). Genome size was highly variable in the two Austrian populations (genome sizes ranging from 414 to 792 Mbp), whereas clones isolated from Lake Nakuru sediments differed little from each other (426 ± 5.1 Mbp, mean and standard deviation). The two clones from the Mongolian site had distinct and relatively large genome sizes of 652 and 732 Mbp (Data from [32]).

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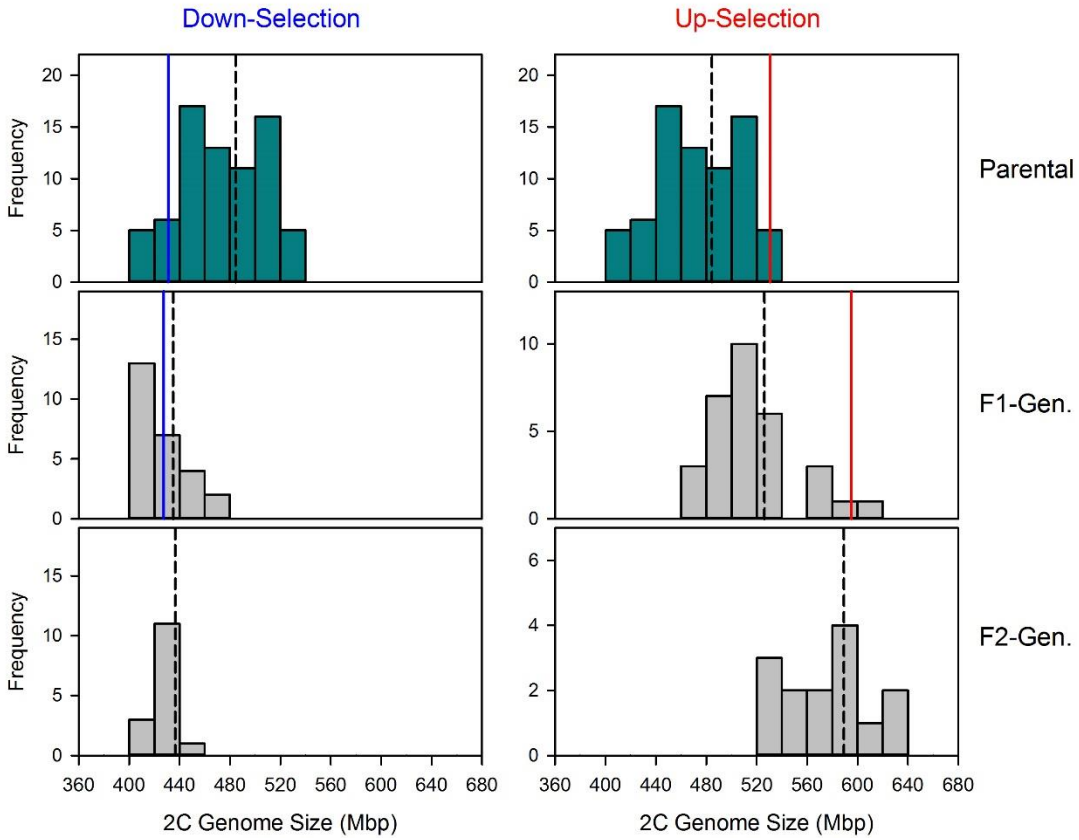
Fig. 2



452

453 **Fig. 2** Artificial selection for increased or reduced genome size. The parental population consisted of
454 clones isolated from the natural population from Obere Halbjochlacke (cf., Fig. 1a) and was the same
455 for both treatments. Dashed black lines indicate population mean genome sizes for each generation.
456 Solid lines indicate the mean genome size of the selected top 10% (in red) or bottom 10% (in blue)
457 for the parental and F1-generation.

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Fig. 3

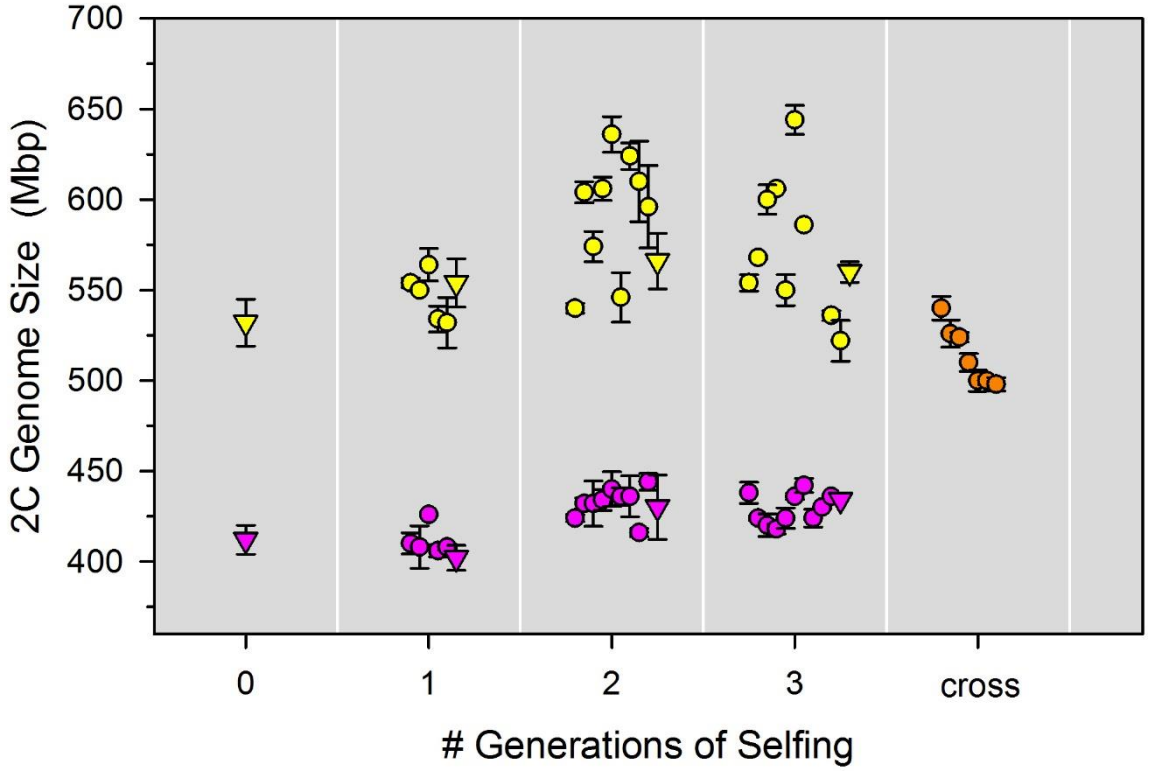

Fig. 3 Genome size variation in two selfed lines and their inter-line cross. Two selfed lines were established from two natural rotifer clones, one with large genome size (524 Mbp, yellow) and one with small genome size (414 Mbp, purple), and were propagated for three sexual generations. Clones used for breeding are indicated by triangles, their siblings are displayed as circles. After three generations of selfing, the two lines were crossed with each other to produce hybrid offspring (orange).

Fig. 4

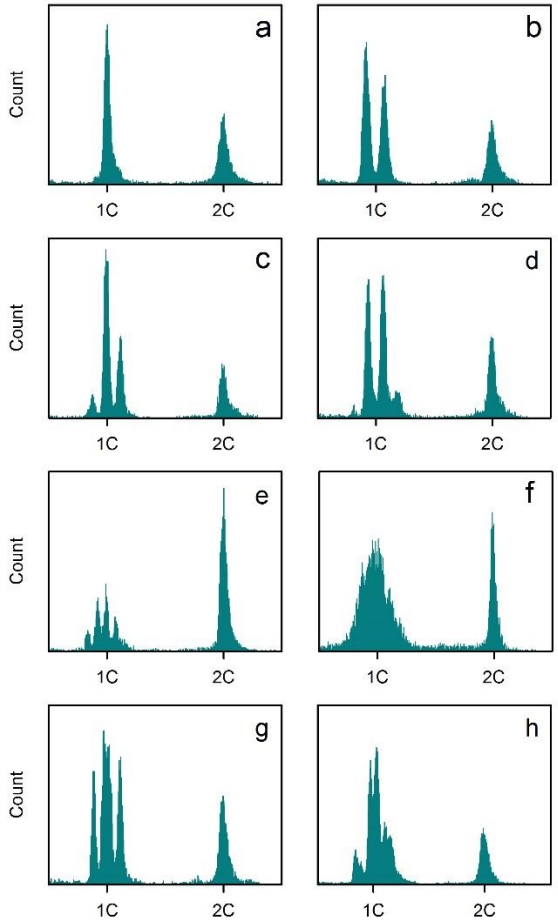

Fig. 4 Representative examples of genome size variation in haploid males. Each histogram corresponds to a different clone. X-axis corresponds to the YL2-A fluorescence on a linear scale. Only the section surrounding the 1C and 2C values is shown. The 1C value was set at 0.5x the median fluorescence of the 2C (female) peak. **a** One single "male peak" (MP), indicates that male genome size is not variable. Note that the MP is located at exactly one half (=1C) of the diploid female genome size (2C). **b** Two MPs located at 0.46 and 0.54 of female genome size, indicating that there are two discrete classes of male genome size. **c** Three MPs, located at 0.44, 0.5, and 0.56. **d** Four MPs, located at 0.41, 0.47, 0.53, and 0.59. **e** Five MPs, located at 0.42, 0.46, 0.5, 0.54, and 0.58 (the MP on the far right is very faint). **f** No discrete MPs, but extremely broad distribution of male genome sizes around the 1C value. However, the 2C peak of this sample is very narrow (~2% CV), indicating that the broad MP is due to real variation in genome size rather than a lack of precision. **g** Four MPs. Note that in contrast to **d**, the MPs are approximately in the same height (i.e., relative frequency), and that there is unequal spacing between the MPs (the middle MPs are close together). **h** Six MPs with unequal spacing, showing some resemblance to the case of three MPs, but each MP appears to be split up into two sub-peaks.

Fig. 5

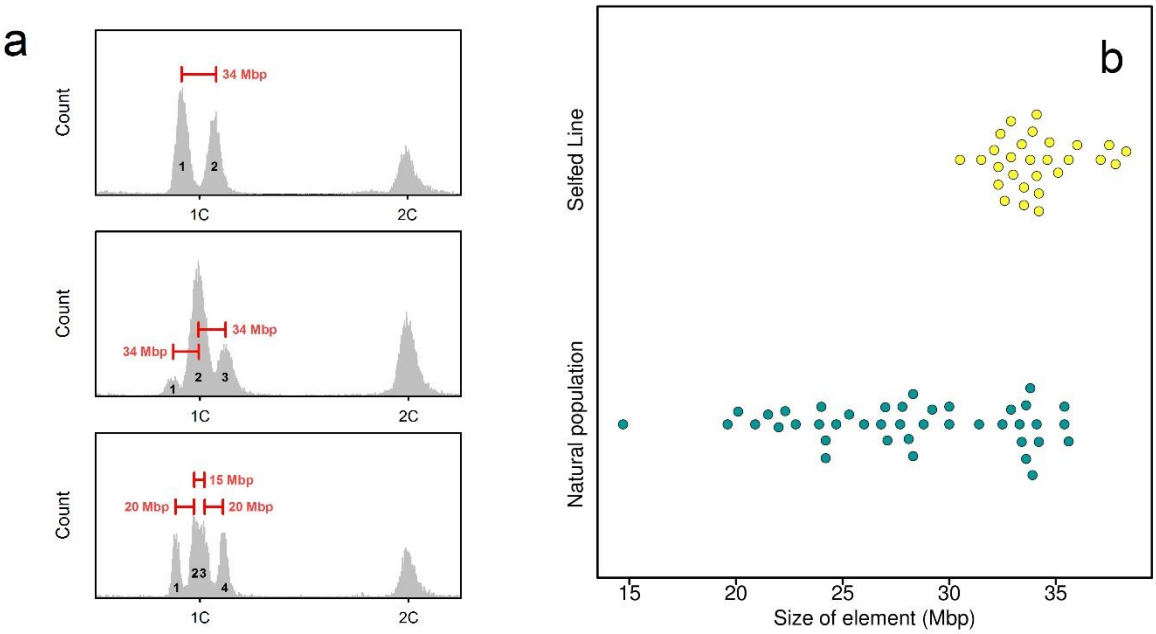

Fig. 5 Sizing of genomic elements responsible for male genome size variation. **a** Three examples of size inference, based on the distance between two male peaks (MPs) and knowledge of diploid genome size. Top: Example of a clone with two MPs, which can be explained by segregation of one ~34Mbp element. Males corresponding to peak 2 contain the 34 Mbp element, while males corresponding to peak 1 are lacking it. Middle: Example of a clone with 3 MPs, which can be explained by two elements of 34Mbp size: Males corresponding to peak 1 are free of elements, males of peak 2 contain one element, and males of peak 3 contain both elements. Bottom: A more complicated case with two, apparently differently sized elements (20Mbp and 35Mbp): Peak 1 corresponds to males without any element, peak 2 to males with the 20Mbp element, peak 3 to males with the 35Mbp element, and peak 4 to males with both elements. **b** Beeswarm plot showing the estimated sizes of elements in the natural population (including their outbred offspring) and in the selfed line (yellow).

Fig. 6

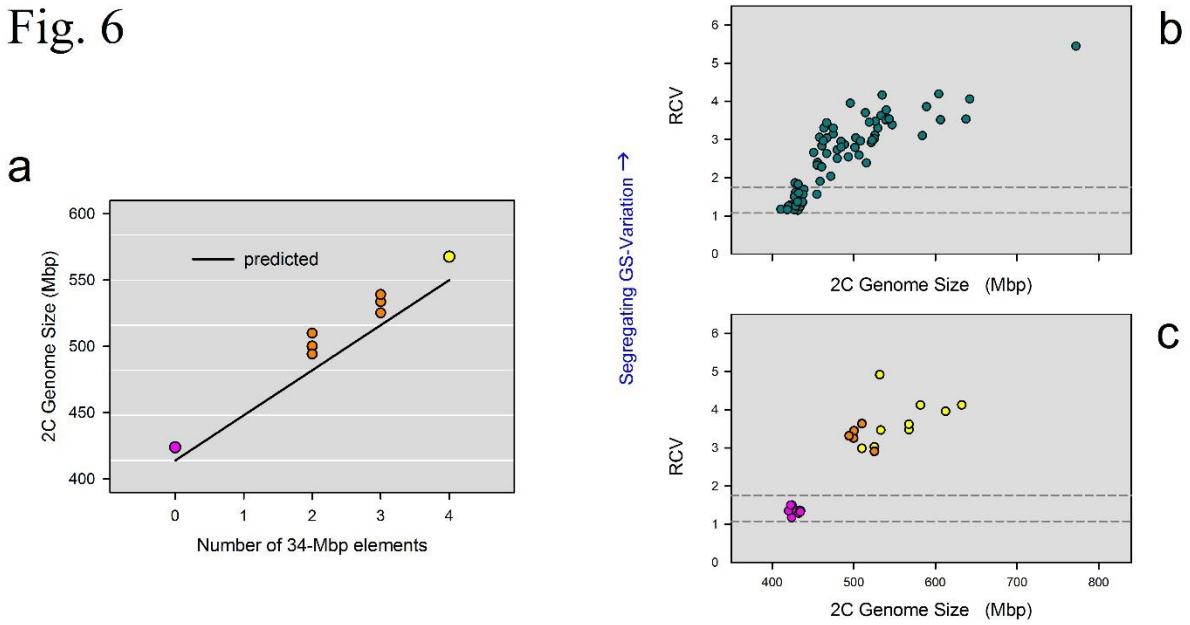

Fig. 6 Mechanistic explanations of intraspecific genome size variation. ***a*** Prediction of genome size based on the size and number of independently segregating elements, assuming a minimum genome size of 414 Mbp (i.e., free of elements). This figure shows seven clones of the inbred line cross (orange), from which we could determine the exact number of 34Mbp-elements based on their MP patterns. The two parental clones, one with four elements (yellow) and the other with zero elements (purple), are also shown. **b** Genome size *vs*. "relative coefficient of variation" (RCV) in clones of the natural population (Fig. 1a) and their outbred descendants. We used RCV as a proxy for the amount of genomic material that independently segregates during meiosis. RCV was calculated by dividing the CV of all combined male genome sizes (in a clone) by the CV of the female genome size. Horizontal dashed lines are the 95% confidence intervals of the RCV in four outgroup species, which have no intraspecific genome size variation (*B. rotundiformis*, *B. plicatilis*, *B. manjavacas*, B. 'Nevada'). **c** Genome size *vs*. RCV in selfed lines. Selfed line with small genome size in purple, line with large genome size in yellow, and the cross between the two inbred lines in orange.

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641 # Summary of all supplementary information

642 **Supplementary information**

643 Supplementary Figures 1–11, Supplementary Table 4, and Supplementary Methods.

644

645 **Supplementary file**

646 Supplementary table 1: Genome size estimates of all clones used in this study; 2: Clones used for
647 sizing of independently segregating elements; 3: Clones used for RCV (relative coefficient of variance)
648 calculations.

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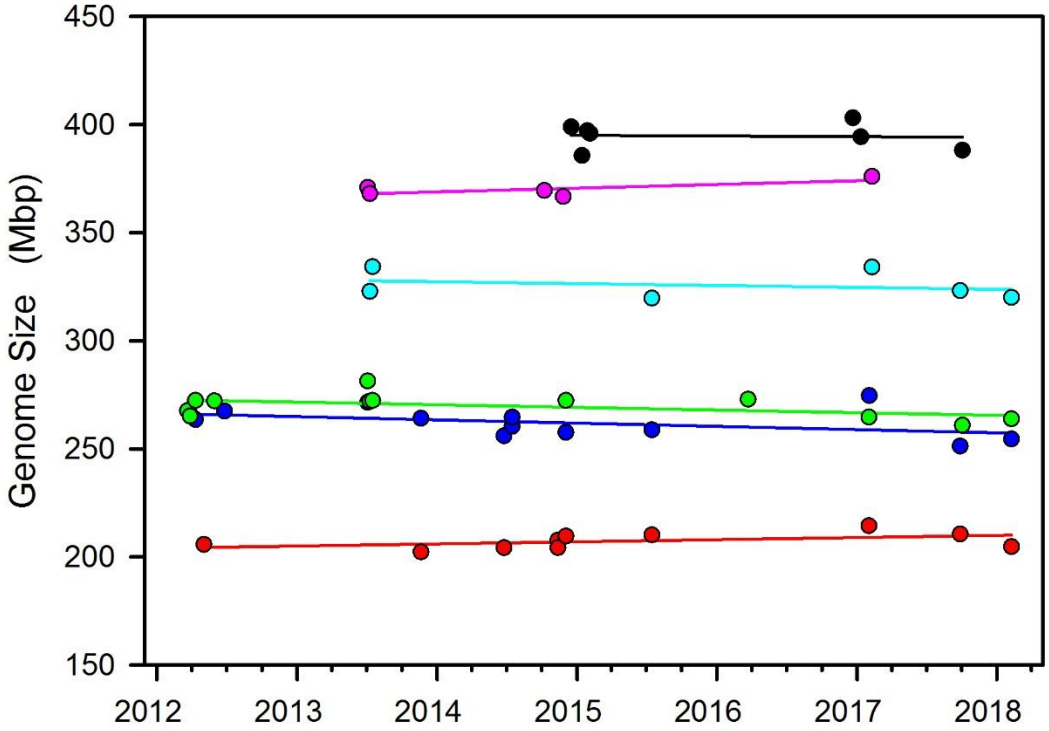
We thank Alois Herzig for providing access to the sampling sites and help during sampling of Obere Halbjochlacke and Runde Lacke. Sediments from Lake Nakuru were kindly supplied by Steve Omondi and Michael Schagerl. Julie Blommaert helped in preparation of media in some of the experiments. We thank David Mark Welch and Julie Blommaert for helpful comments on the manuscript.

Authors' contributions

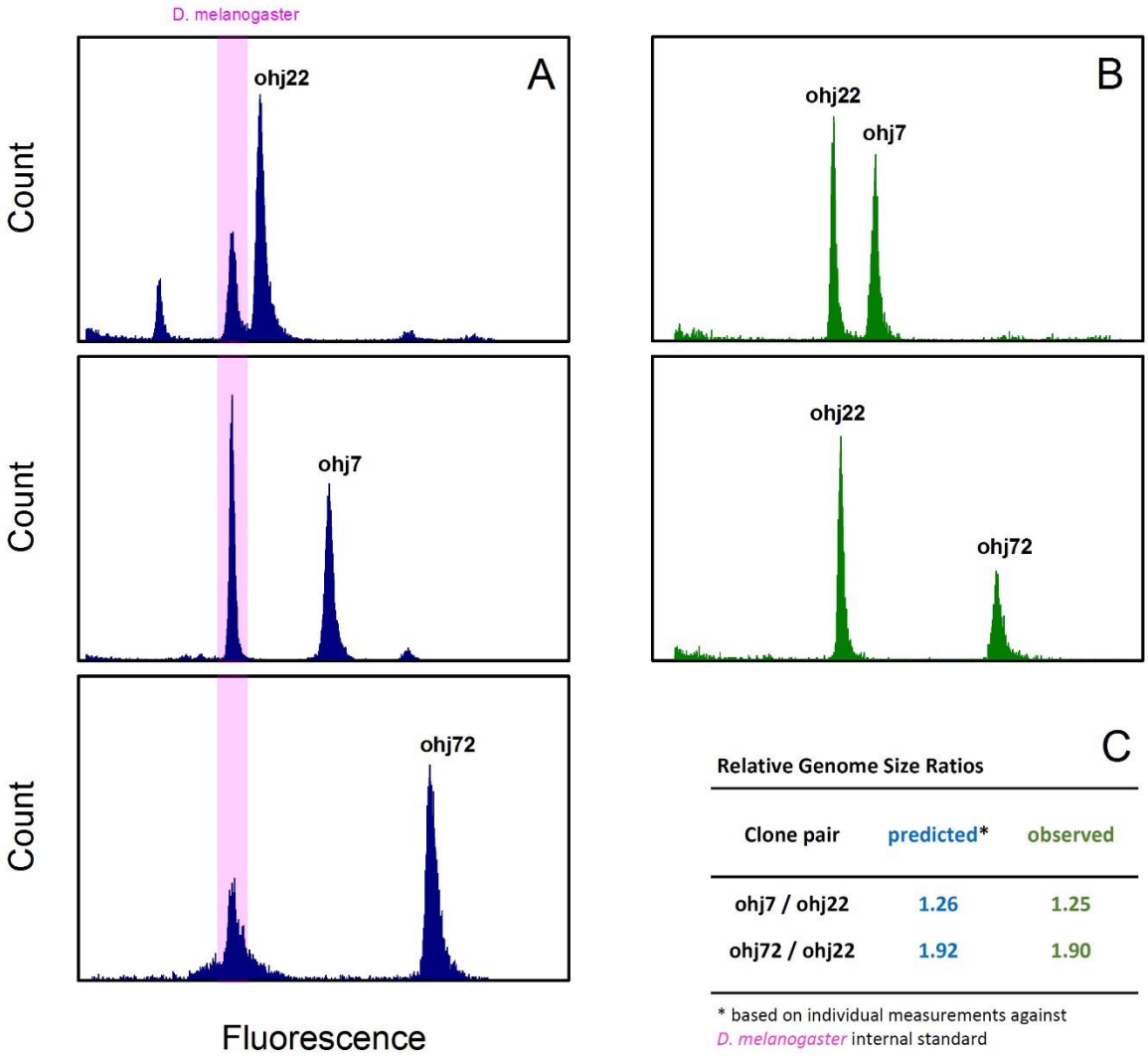
CPS conceived the study, designed and participated in all experiments. MP and PS contributed the flow-cytometry analysis. SR contributed the line crosses and the selection experiment. AH and MP did most of the culturing work and prepared rotifer biomass for flow cytometry. CPS analysed the data and drafted the manuscript. All authors read and approved the final manuscript.

Supplementary Information

Supplementary Figures



Supplementary figure 1. Long-term constancy of genome size during clonal propagation. Time courses of genome size for six *B. asplanchnoidis* clones over a period of > five years (i.e., approximately 600 asexual generations). Colours denote different clones: *ohj22* (red), *ohj7* (blue), *ohj13* (green), *mnchu24* (cyan), *mnchu008* (pink), *ohj72* (black). Each symbol represents an independent genome size measurement.



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676 **Supplementary figure 2. Comparison of two variants of the flow cytometry method for measuring**
677 **intraspecific genome size variation. a** Three clones of different genome size were co-prepared with
678 an internal standard of known genome size (*D. melanogaster*, strain ISO 1, 352 Mbp). **b** Pairs of the
679 same three clones were combined into a sample. **C** A comparison of the genome size ratios (= large
680 GS / small GS) determined by the second method shows that these closely matched those predicted
681 by the measurements of the individual rotifer clones, as determined by the first method.

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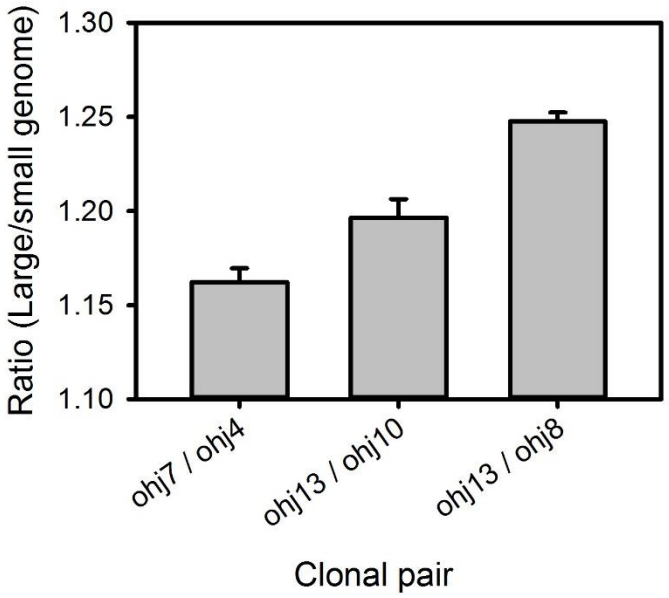

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684 **Supplementary figure 3. Genome size differences among selected pairs of clones in the OHJ-**
685 **population.** Error bars are standard deviations based on 3-4 replicates (measured on different dates).
686 In each replicate the clonal pairs were co-prepared and stained in the same tube. Kruskal-Wallis test:
687 *H*= 8.95, *d.f.*=2, P=0.011.

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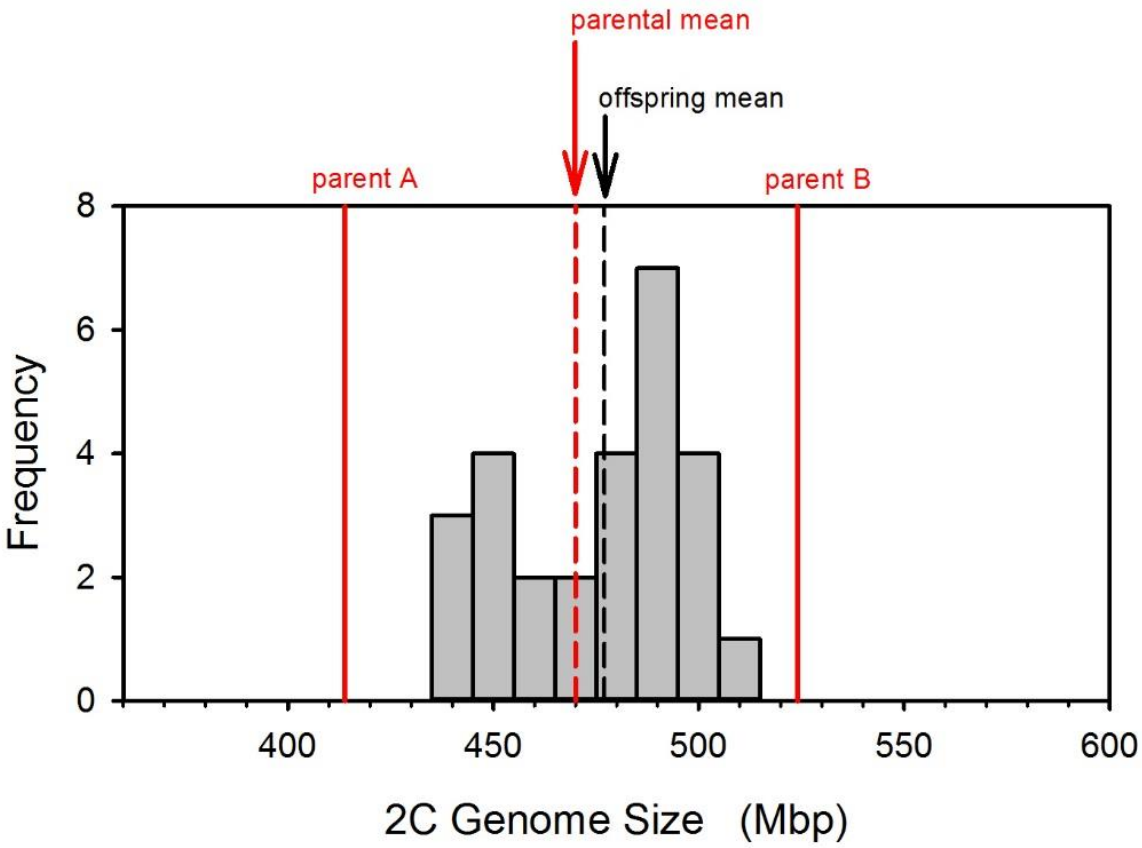
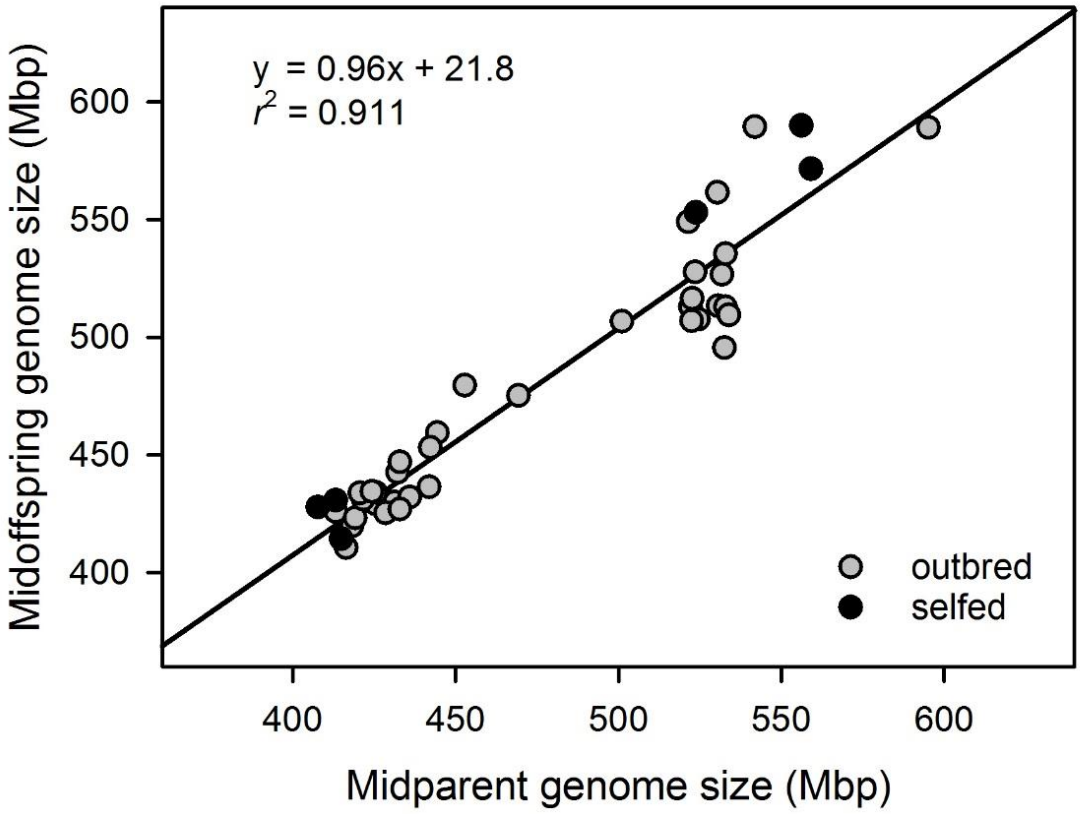
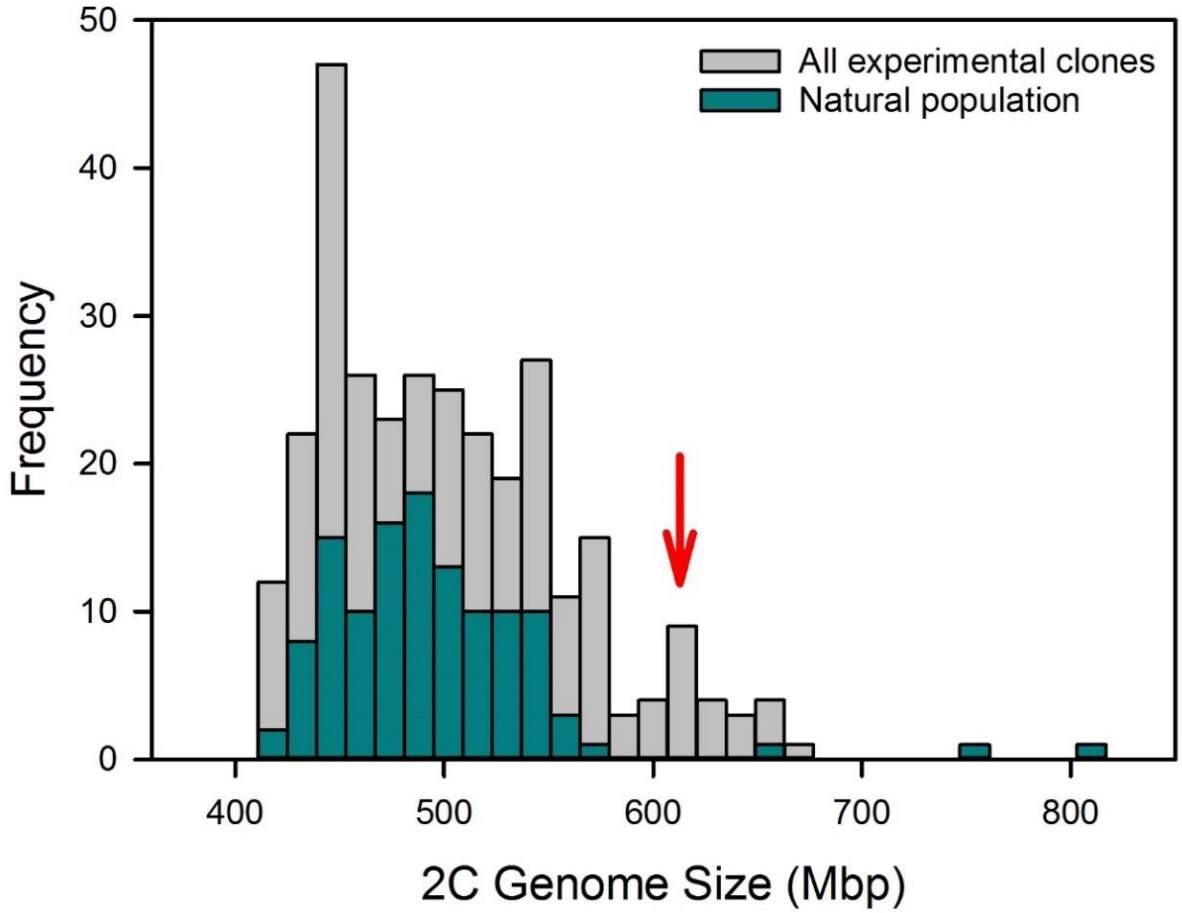


Supplementary figure 4. Sexual cross between a clone with large genome size and a clone with small genome size. Parental genome sizes (414 and 524 Mbp) are indicated by the vertical red lines. The dashed red line indicates the parental mean, while the dashed black line indicates the offspring mean genome size. Overall, the 27 offspring genome sizes were intermediate between their parents, even though their distribution was not unimodal.



Supplementary figure 5. Narrow-sense heritability (h^2) of genome size in *B. asplanchnoidis*. Each symbol represents the mean of parents and offspring genome sizes of a family. This figure combines our results from the artificial selection experiment and crossings between clones with large and small genome size.



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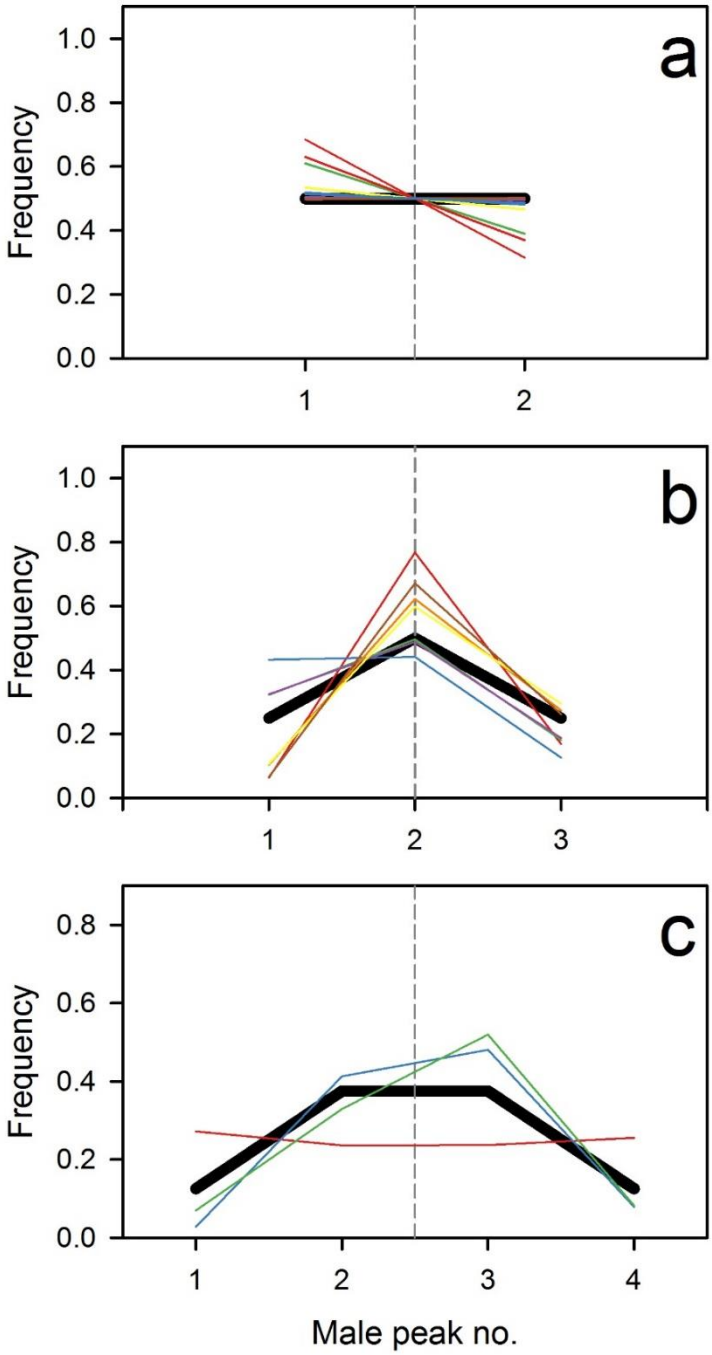
708 **Supplementary figure 6. Distribution of genome sizes found in *B. asplanchnoidis*. Gray**: All *B.*
709 *asplanchnoidis* clones measured so far (including natural populations, selection lines, and selfed
710 lines). **Green**: Only the natural OHJ population. **Red arrow** highlights genome size classes absent in
711 the natural population, which could be artificially selected in the laboratory.

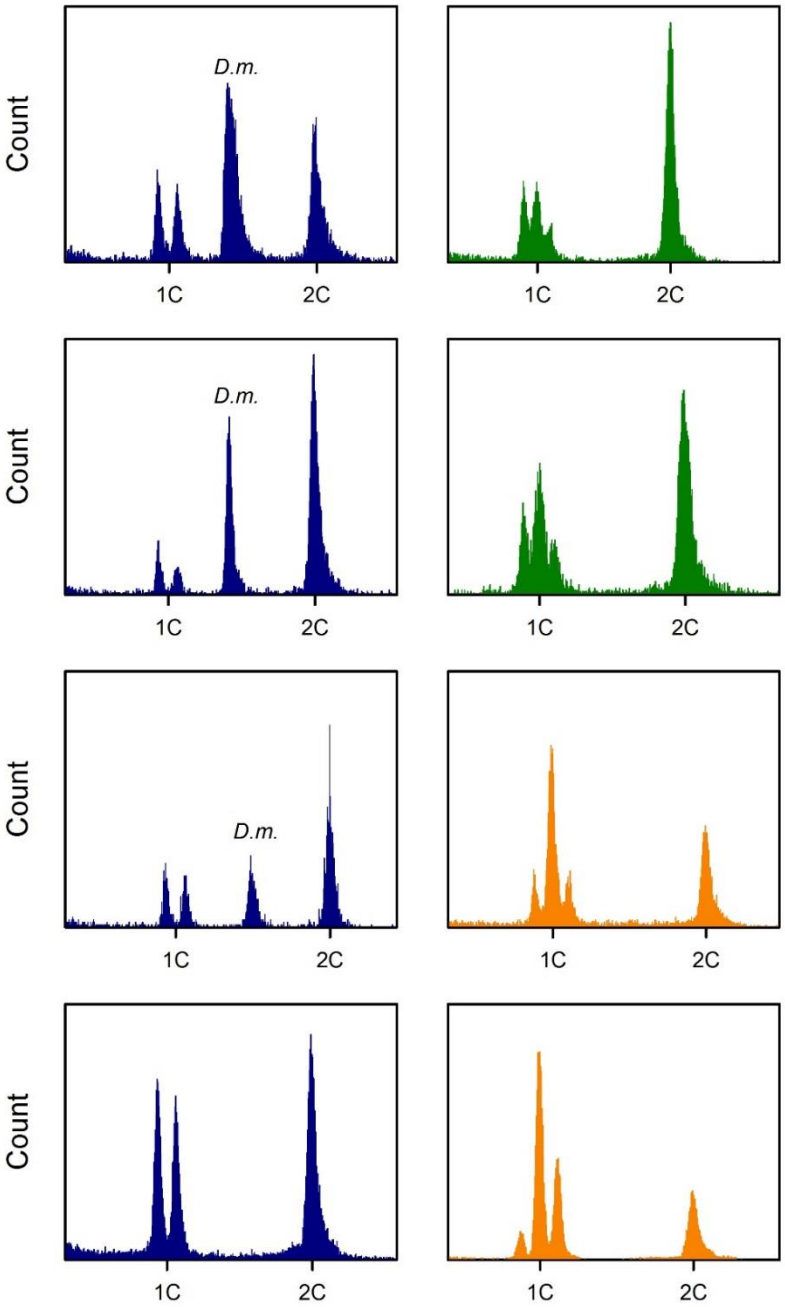
712



713

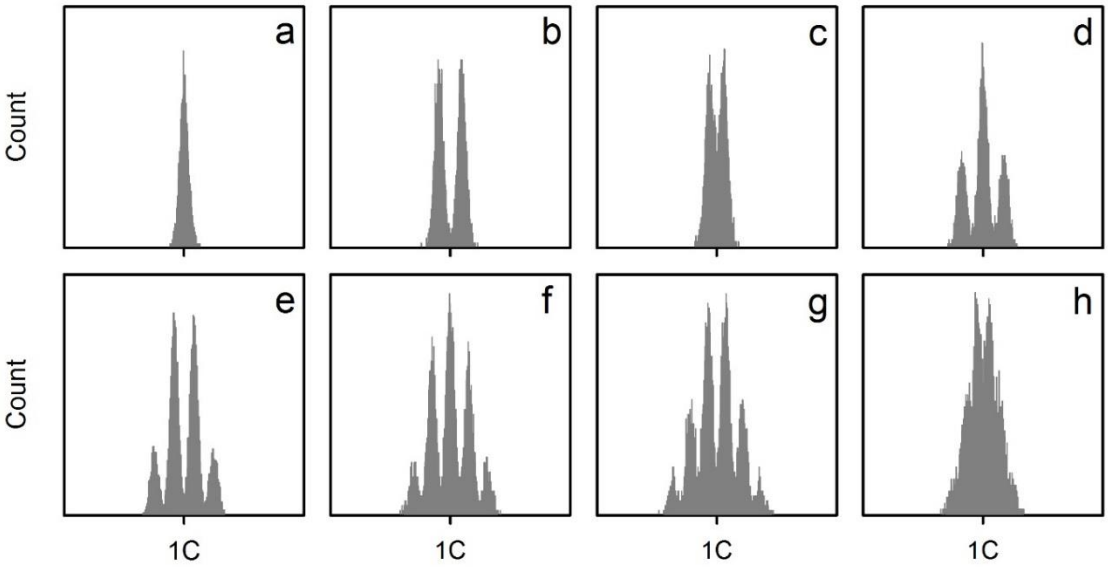
714

715 **Supplementary figure 7. Relative frequency of the different male genome sizes. a** two malepeaks;
716 prediction (thick line) based on one independently segregating element **b** three malepeaks;
717 prediction based on two equally sized elements, **c** four male peaks; prediction based on three equally
718 sized elements. Coloured lines denote different clones. Dashed vertical line indicates 0.5x diploid
719 genome size.

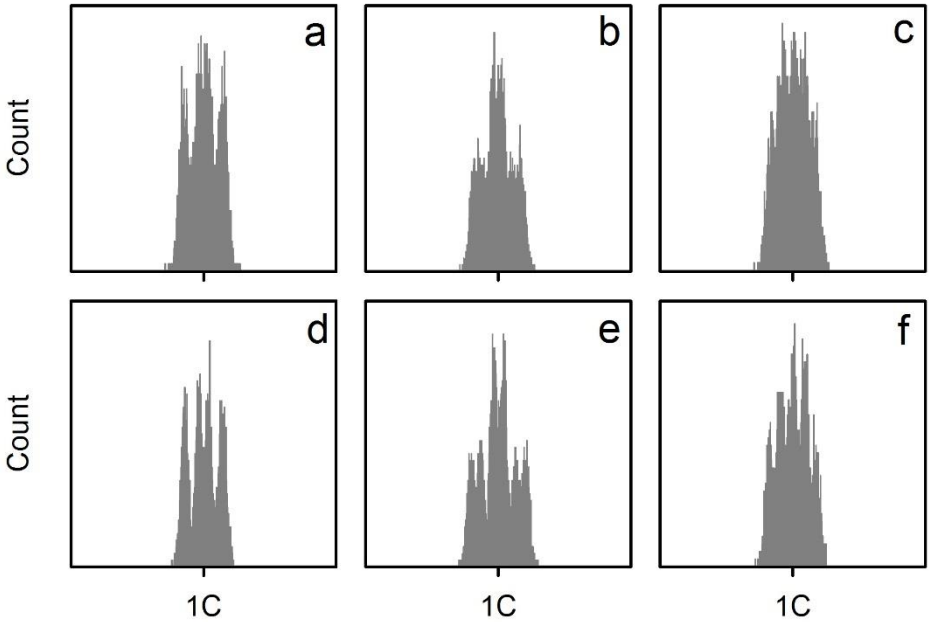


720

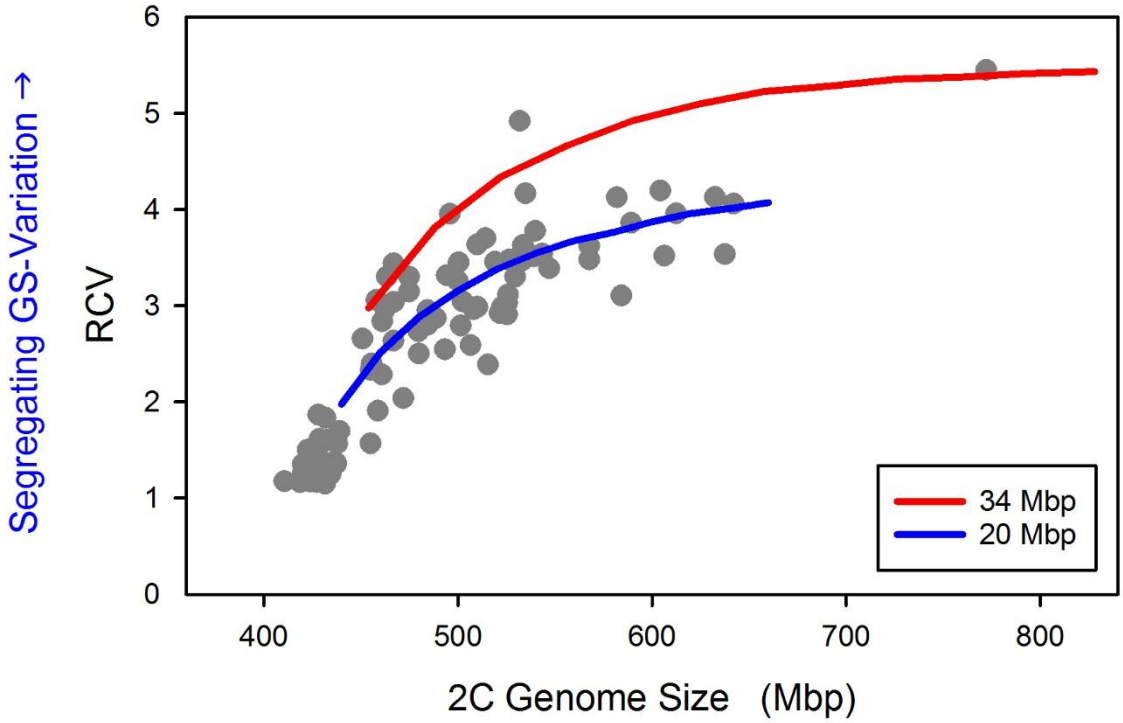
721 **Supplementary figure 8. Constancy of "male peak" (MP) patterns in asexually propagated clones.**
722 The number of male peaks and their heights (relative to each other) are characteristic for a clone.
723 This figure shows the 2C-female peaks and the corresponding male peaks (peaks scattering around
724 1C) for three different clones on different dates. **Blue**: Clone *k13x110n2* (two MPs); **Green**: Clone
725 *ohj76* (three MPs); **Orange**: Clone *ohj85* (three MPs). In some of these measurements we used
726 *Drosophila melanogaster* (**D.m.**) as internal standard. Please note that the height of MPs relative to
727 the 2C-female peak might differ among dates, since the number of males per female in a culture is
728 not always the same.



Supplementary figure 9. Model simulations of "male peak" (MP) patterns with only one type of independently segregating element. a No element; **b** one element of 34 Mbp size; **c** one element of 20 Mbp; **d** two elements of 34 Mbp; **e** three elements of 34 Mbp; **f** four elements of 34 Mbp; **g** five elements of 34 Mbp; **h** five elements of 20 Mbp. The model-parameter 'measurement precision' was set to 2.7% (i.e., the coefficient of variance) in all cases.



Supplementary figure 10. Model simulations of "male peak" (MP) patterns with different types of independently segregating elements. a two elements with 20 and 34Mbp; **b** three elements with 15, 34, and 34 Mbp; **c** three elements with 15, 20, and 34 Mbp. The model-parameter measurement precision was set to 2.7% (i.e., the coefficient of variance). **d-f** are identical to **a-c**, except that measurement precision was set to 2.0%.



Supplementary figure 11. Model simulations of the relationship of RCV and genome size. RCV ("relative coefficient of variation") was calculated by dividing the CV of all combined male genome sizes (in a clone) by the CV of the female genome size. Red line shows model predictions for genomes with multiple 34 Mbp elements. Blue line shows model predictions for genomes with multiple 20 Mbp elements. . The model-parameter 'measurement precision' was set to 2.7%. Dots are all measurements of RCV in this study (cf. Fig. 6 b & c)

757 ## Supplementary Tables

758

759 **Supplementary table 4.** Examples of natural intraspecific genome size variation in animals and plants

Species name	Common name	1C-value (pg)	Fold-variation in GS	Reference
Zea mays	Maize	2.30	1.37	Diez et al. (2013)
Festuca pallens	Grass	2.53	1.19	Smarda et al. (2008)
Microseris douglasii	Coastal silverpuff	1.20	1.20	Smarda & Bures (2010)
Hordeum spontaneum	Wild barley	5.50	1.13	Smarda & Bures (2010)
Arabidopsis thaliana	Thale cress	0.16	1.14	Long et al. (2013)
Eyprepocnemis plorans	Grasshopper	10.39	1.19*	Ruiz-Ruano et al. (2011)
Synalpheus duffyi	Snapping shrimp	17.38	1.35	Jeffery et al. (2016)
Drosophila melanogaster	Fruit fly	0.18	1.15†	Huang et al. (2014)
Brachionus asplanchnoidis	Rotifer	0.21	1.33 (1.91)	**this study**

* assuming individuals with zero *vs*. six B-chromosomes of 0.64pg size

† variation between inbred strains

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766 ## Supplementary Methods

767

768 ### Rotifer cultures

769 Rotifers were cultured in F/2 medium [46] at 16 ppt salinity and with *Tetraselmis suecica* algae as
770 food source (500–1000 cells μl^{-1}). Continuous illumination was provided with daylight LED lamps
771 (SunStrip, Econlux) at 30–40 µmol quanta m^{-2} s^{-1} for rotifers, and 200 µmol quanta m^{-2} s^{-1} for algae.
772 During the experiments, rotifers were cultured at 23°C. Stock cultures were kept either at 18 °C, re-
773 inoculated once per week by transferring 20 asexual females to 20ml fresh culture medium, or they
774 were kept for long-term storage at 9°C, replacing approximately 80% of the medium with fresh food
775 suspension every 4 weeks.

776

777 ### Genome size measurements

778 To determine the genome size of rotifer clones, we used flow cytometry and propidium iodide
779 staining of nuclei. Rotifer clones were grown in 250-1000 ml flasks until their population size reached
780 several hundreds to a few thousand individuals. On the day before preparation, rotifers were
781 harvested with 60 µm sieves, and starved overnight in 11 ppt 0.2µm-filtered medium, followed by
782 two washes with filtered medium on the next day. For each sample, we isolated 350 females and
783 subjected them to the flow cytometry protocol. Briefly, rotifers were homogenized in a citrate buffer
784 (3.4 mM Trisodium citrate dihydrate, Nonidet P40 at 0.1% v/v, 1.5 mM Sperminetetrahydrochloride,
785 0.5 mM Trishydroxymethylaminomethane, pH 7.6), and were then transferred to 750 µl stock
786 solution in a 1 ml Dounce tissue homogenizer. Rotifers were homogenized on ice with 20 strokes
787 using the "tight" pestle of the homogenizer. As internal standard of known genome size we used the
788 fruit fly, *Drosophila melanogaster* (strain ISO-1, nuclear DNA content: 0.35 pg, [45]). After adding two
789 female *Drosophila* heads, the sample was further homogenized with ten strokes. Large debris was
790 removed by filtration through a 40 µm mesh nylon sieve. After addition of 100 µl of 0.021% Trypsin
791 (dissolved in stock solution) the sample was incubated for exactly 10 min at 37°C. To prevent further
792 degradation, 75µl of 0.25% trypsin inhibitor was added (this solution also included 0.05% RNAse A)
793 and the samples were incubated for another 10 min at 37°C. Finally, samples were stained with
794 propidium iodide at a concentration of 50 µg/ml. Stained samples were kept overnight on ice in the
795 dark. Flow cytometric analysis was performed on the next day on an Attune NxT® acoustic focusing
796 cytometer (Thermo Fisher) with an excitation wavelength of 561 nm and a custom-made 590–
797 650 nm bandpass filter (yellow, YL-2) for detection of propidium iodide fluorescence. Flow cytometric
798 data were analyzed using FlowJo software version 10.0.7r2 (FlowJo LLC). To exclude doublets (i.e.,
799 nuclei that pass the detector too close together, thus being recorded as a single "event") we

800 employed YL2-A *vs*. YL2-H gating. Coefficients of variance (CVs) of individual peaks typically ranged
801 between 1.5% and 4% for both *Drosophila* and rotifers. Very few measurements had CVs higher than
802 5%, and those replicates were discarded. Conversion from picograms DNA to base pairs were made
803 with the factor: 1 pg =978 Mbp [45]. In most cases, we obtained at least three replicate
804 measurements for a clone, usually on different days and sometimes with several weeks or months
805 apart.

806

807 ## Crossings between rotifer clones

808 For sexual crosses between two rotifer clones, we used freshly hatched virgin females and males,
809 which were harvested as eggs from dense rotifer cultures that had initiated sexual reproduction. Eggs
810 were detached from the females by vigorously vortexing the rotifer culture in 50-ml Falcon tubes for
811 ten minutes. Crossings between clones were accomplished by placing 100 female eggs and 50 male
812 eggs together into the same well of a 24-well plate filled with 750 µl of F/2 medium. After 24 h, when
813 all viable eggs had hatched and animals had time to mate, females were transferred to new wells
814 with fresh food suspension. After a few days, when females started producing eggs, we classified
815 them into asexual females, sexual male-producing females (which were unfertilized), and sexual
816 resting egg producing females. All resting egg producing females were isolated and stored at 7 °C in
817 the dark for at least 2 weeks. To induce hatching, resting eggs were incubated with food suspension
818 at 23 °C and high light intensities (200 µmol quanta m^{-2} s^{-1}). Usually after 48 h, the first hatchlings
819 started to emerge, and clonal cultures were initiated.

820

821 ## Theoretical model of independently segregating elements

822 We conceptualized a theoretical model to summarize our finding of independently segregating
823 genomic elements. The main purpose of this model was to test the hypothesis that these genomic
824 elements are *sufficient* as the mechanism of genome size variation. In particular, we examine
825 whether such a model is consistent with our observations of genome size variations in males from
826 clones of different diploid genome size. The basic input parameters and variables of the model are:
827 (i) an assumed minimum diploid genome size of 414 Mbp (for a clone that contains no additional
828 genomic elements), and (ii) a vector describing the size and number of individual elements in a clone
829 (e.g., [34 34 20] for two 34 Mbp and one 20 Mbp element, respectively). The output variable of the
830 model was the predicted diploid genome size of females, calculated as minimum genome size + the
831 summed contribution of all elements. The second output variable was the genome size distribution
832 of haploid males of this same clone (i.e., its "male-peak pattern"). Male genome sizes were

calculated as ½ minimum genome size+ the summed contribution of all elements in a particular male. To this end, we assumed that all elements segregate completely independently from each other during meiosis, i.e., each element having an equal chance of ending up in one out of four gametes after the two meiotic divisions. For instance, a clone with two 34 MBp elements should produce 25% gametes/males containing no element, 50% gametes containing one element (50%), and 25% containing two elements. We calculated the male genome size distributions for a total of 10,000 gametes/males per clone. We also simulated measurement errors by assuming a coefficient of variance of 2.7%. This value is based on the average precision of our flow-cytometry measurements of male genome size variation (Supplementary table 3). We also explored CV values of 2%, which we obtained in some of our best samples. The model was written in the MATLAB programming environment (MathWorks®, version R2017A) with the code as follows.

```
function [relCV, gsFem, allGametes, allGametesERR] = ...
    estimateRelativeCV(Bvec, measErr)

% MATLAB FUNCTION "estimateRelativeCV" for calculation of (diploid) female
% genome size and male genome size distribution, based on a vector
% specifying the size and number of independently segregating elements in a
% clone.
%
% Claus-Peter Stelzer (2019)

% Input variables
% Bvec        = [20]; % Vector of elements (contains size in MBp)
% measErr   = 2.7; % measurement error in flow cytometry (coefficient of
%                    variance in %)

% Model parameters
baseGS      = 207;                  % Genome size of male without elements
gsFem       = baseGS*2 + sum(Bvec); % Genome size of diploid female
nGametes   = 10000;                 % Number of gametes/males simutated
allGametes = nan(nGametes,1);

for i = 1 : 2 : nGametes-1
    GameteA = baseGS;
    GameteB = baseGS;
    for j = 1:length(Bvec)
        if rand>0.5
            GameteA = GameteA + Bvec(j);
        else
            GameteB = GameteB + Bvec(j);
        end
    end
    allGametes(i)   = GameteA;
    allGametes(i+1) = GameteB;
end

% Convert coefficient of variance into standard deviation
maleMeanGS = gsFem/2;
sdMales = measErr/100*maleMeanGS;
```

```
% Simulate with measurement error
allGametesERR = allGametes + sdMales*randn(size(allGametes));
mpCV = std(allGametesERR)/mean(allGametesERR)*100;
relCV = mpCV/measErr;
```