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 the maintenance and evolution of such variation on a population level. 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 (multiple Mega-Base pairs in size) provide the substrate of genome size variation. These elements explain the short-term evolutionary dynamics of genome size as well as unexpected patterns, like increased genome size variation among inbred lines. Overall, our study explains how intraspecific genome size variation can be maintained in populations and provides a framework for understanding the evolutionary forces that drive changes in genome size on a population level.

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, and that having more DNA in the genome can sometimes shift the phenotype to a more optimal state (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, 14).

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 distantly related species, which 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 (15). 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 (15, 16). 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 (17), with cultivated maize and its close relatives being probably one of the best-studied examples (18, 19). In animals, intraspecific genome size variation has been found in snapping shrimp (20) and in grasshoppers (21). 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 (22, 23), suggesting that his phenomenon might be more widespread than previously assumed. However, despite its importance, surprisingly

67 little is known about how intraspecific genome size variation is maintained in natural populations,
68 how it is inherited to offspring, and how fast the trait 'genome size' can evolve. Even in the best-
69 studied systems, researchers typically rely on assumptions. For example, genome size is often
70 modelled like a quantitative trait (24, 25), despite a lack of knowledge about the number and
71 contribution of individual loci.

72 Here we study the mechanisms and dynamics of genome size variation in a population of the
73 rotifer *Brachionus asplanchnoidis*. This rotifer is characterized by an elevated genome size relative to
74 its sister species, and by intraspecific genome size variation (26, 27). Using crossbreeding
75 experiments, selfed lines, and artificial selection, we disentangle how variation for genome size is
76 maintained in this population, and how it is inherited to offspring. We capitalize on several
77 advantages of our model system, such as short generation times, sexual and asexual reproduction,
78 and a haploid-diploid lifecycle, which allowed us to probe into meiotic patterns associated with
79 intraspecific genome size variation. Thus, we could identify the size and number of individual
80 elements that contribute to increases in genome size. Our findings explain how genome size variation
81 is maintained at a population level, and suggest several general features of intraspecific genome size
82 variation and their link to microevolutionary change in genome size.

83

84 # Results

85

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

87 To quantify genome size variation within populations, we examined 118 *B. asplanchnoidis* clones
88 sampled from four geographic populations (Fig. 1, Supplementary table 1). Of these, two Austrian
89 populations from 'Obere Halbjochlacke' (OHJ, 74 clones) and 'Runde Lacke' (RL, 29 clones) show
90 highly significant intrapopulation variation (OHJ: ANOVA $F_{52,194} = 112$, $P < 0.001$; RL: $F_{28,84} = 23.14$, $P <$
91 0.001). The OHJ-population spans a genome size range of 1.33-fold, from 414 to 552 Mbp (Fig. 1a),
92 with one outlier at 792 Mbp (i.e., 1.91-fold). The RL-population is also variable (Fig. 1b), spanning a
93 range of 1.24-fold across all sampled clones. In contrast, the Lake Nakuru population (11 clones
94 sampled) was not significantly variable ($F_{10,21} = 8.64$, $P = 0.078$), and most genomes were close to 420
95 Mbp (Fig 1c). Previously, we reported two conspecific clones isolated from a Mongolian lake, which
96 have relatively large genome sizes of 652 and 732 Mbp (Fig. 1c, data from Riss et al. 2016). Genome
97 size is mitotically stable, since the genome sizes of clones, as well as the differences among clones,
98 were highly reproducible over a period of more than five years (i.e., approximately 600 asexual
99 generations, Supplementary Fig. 1).

100 Claims of intraspecific genome size variation have sometimes met scepticism, due to possible
101 methodological artefacts (28), or the species concept underlying the study (29). Thus, we used two
102 independent flow-cytometric methods, both involving internal controls, either *Drosophila*
103 *melanogaster* flies or other rotifer clones that were co-prepared with a focal sample. Both methods
104 yield virtually identical results, confirming that our genome size estimates are accurate and reflect
105 real differences among clones (Supplementary Figs. 2, 3). Previous studies have documented the
106 species status of *B. asplanchnoidis* (30, 31), and have shown that the populations from Austria,
107 Mongolia and Lake Nakuru are genetically distinct, but also experience natural gene flow on a larger
108 geographic scale (31). Thus, in the case of *B. asplanchnoidis* we can exclude any unrecognized cryptic
109 species.

110

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

112 Clones with divergent genome size can mate with each other and produce viable and fertile
113 offspring, which are intermediate in genome size between their parents but show some variation
114 (Supplementary Fig. 4, see also (31)). Genome size responds to artificial selection with extremely high
115 heritability. We applied truncation selection to the OHJ population by crossing clones with the 10%

largest genome sizes among each other (excluding the outlier clone at 792Mbp), and by crossing clones with the 10% smallest genomes, respectively. In the up-selection treatment, we obtained genome sizes exceeding the range of the parental OHJ population (Fig. 2). We could select genome sizes of up to 640 Mbp, with a narrow-sense heritability h^2 of 0.905 in the first generation, and 0.912 in the second generation. Likewise, heritability was high in the first generation of the down-selection treatment (h^2 = 0.924). However, in contrast to selection for large genome size, it did not extend the range of the original population. In fact, we could not select genome sizes below 414 Mbp. Additionally, h^2 of the second generation of the down-selection treatment collapsed to zero. A parent-offspring regression including all our crosses yields an overall estimate for h^2 of 0.96 (Supplementary Fig. 5).

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

Inbred lines can provide additional insights into the inheritance of a trait. Theoretically, one would expect trait variation to decrease upon selfing as this causes 50% reduction of heterozygosity each generation. In contrast, we found that genome size variation remained high in a selfed line that descended from a large-sized clone (524 Mbp). Genome sizes of selfed offspring ranged between values of 522 Mbp and 644 Mbp, representing a 23% increase (Fig. 3). Among-clone variation in the large selfed line was statistically significant, even after three generations of selfing (ANOVA $F_{9,24}$ = 53.61, $P < 0.001$). Variation in the large line was also significantly higher than in the line descending from the small genome ($p < 0.001$; R package *cvequality*, version 0.2.0, (32)). After three generations of selfing, we performed a sexual cross between both lines. Like in the cross between two natural clones, the cross of the selfed lines yielded offspring that were variable and intermediate between their parents (Fig. 3).

These results establish that genome size in *B. asplanchnoidis* is a trait under complete genetic control. At a quick glance, this supports the notion that genome size is a quantitative trait governed by a large number of insertion and deletion alleles (24). However, the fact that genome size variation remained high in the selfed line suggests that a “simple” quantitative trait model might not capture all aspects of the genome size dynamics. Additionally, our finding of a minimum genome size at

150 ~414Mbp seems to be incompatible with a model of many loci with either positive or negative effects
151 on genome size.

152

153 **Evidence for independently segregating genomic elements**

154 To gain mechanistic insights into segregation of genome size variation during meiosis, we
155 analysed haploid rotifer males. In a diploid organism with equally sized chromosome pairs and no
156 extra-chromosomal elements, all meiotic products should contain exactly half the DNA of a diploid
157 cell. Consistently, haploid males of the rotifer *B. calyciflorus* have half the genome size of females
158 ((33); see Appendix S2 in this publication). We also find this pattern in some of our *B. asplanchnoidis*
159 clones (e.g. Fig. 4a). However, in many others we obtained striking variation in male genome size,
160 which manifested in multiple discrete "male peaks" (Fig. 4b-h, Supplementary table 2). In the
161 simplest case, we observed two male peaks spaced symmetrically around the expected 1C-value (Fig.
162 4b). In general, the male peak pattern of a clone could be characterized by (i) the number of peaks,
163 (ii) an odd/even number of peaks, indicating the presence/absence of a central male peak at exactly
164 1C, and (iii) the relative abundance of certain male genome size classes, as inferred from the area
165 under each male peak. In most cases, the central male genome sizes (the ones close to 1C) were
166 more abundant than the ones in the periphery (Supplementary Fig. 7, Supplementary table 2).
167 Overall, male peak patterns were clone-specific and highly repeatable (Supplementary Fig. 8).

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

177 A relatively simple mechanism can explain all the above observations. We hypothesize that
178 large genomic elements, several Mega-base pairs in size, are segregating *independently* from each
179 other, and thus give rise to discrete classes of male genome size. By independent, we mean that each
180 element has an approximately equal chance of segregating into any of the four gametes during
181 gametogenesis. Note that this is different from normal chromosome segregation, where homologous
182 chromosomes will always end up in opposite gametes. The presence of one independently

183 segregating element can explain the simplest pattern of two male peaks (Fig 4b), being present in the
184 large male genome size but absent in the small one. Similarly, two equally sized elements can explain
185 a pattern of three male peaks (Fig. 4c), which correspond to genome size classes with zero, one, or
186 two elements. Likewise, patterns with higher numbers can be explained by n-1 elements. Assuming
187 that independently segregating elements have identical size, we can predict the relative ratios of the
188 male genome size classes to 1:1 (one element), 1:2:1 (two elements), or 1:3:3:1 (three elements).
189 Indeed, some of our clones closely follow these predicted frequencies, whereas others showed some
190 deviations (Supplementary fig. 7). For example, in clones with two male peaks, the smaller male
191 genome size was often at higher frequency than the expected value of 0.5 (Supplementary fig. 7),
192 suggesting that males without an element were more frequent among the hatched males. Likewise,
193 in clones with three and four male peaks, the central male genome sizes were sometimes at a higher
194 frequency than expected. One clone showing four male peaks with almost identical heights
195 (Supplementary fig. 7c) presents a particularly interesting deviation. In this same clone, the two
196 central peaks were closer together (cf. Fig. 4g). The most parsimonious explanation seems to be that
197 this clone carries two differentially sized elements, a small and a large one, and that the four male
198 peaks correspond to: (1) zero elements, (2) the small element, (3) the large element, and (4) both
199 elements. Likewise, two large and one small element can explain the "three double-peaks" pattern in
200 the clone depicted in Fig. 4h.

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

212

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

214 The hypothesis of independently segregating elements offers an *ad hoc* explanation to many
215 patterns in our data. However, does it provide a *sufficient* explanation for within-population genome

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

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

Identifying the exact size and number of independently segregating elements is not possible for the natural OHJ population as a whole, due to the higher diversity of segregating elements in this population (Fig. 5b), and due to limited resolution of male peak patterns in clones containing multiple differentially sized elements (Supplementary Fig. 9). To circumvent these limitations, we

250 tested whether the amount of meiotically segregating genome size variation is correlated with
251 genome size. To this end, we defined the variable RCV (Relative Coefficient of Variation), which is the
252 CV of all combined male genome sizes of a clone divided by the CV of the female genome size. Thus,
253 RCV accounts for differences in measurement precision across samples (indicated by the CV of the
254 female peak), while it requires only that a clone can produce males in order to be measured, thus
255 extending our analyses to many more clones (n=72). We found that the RCVs of OHJ-clones were
256 considerably higher than those of outgroup rotifer species without genome size variation (Fig. 6b),
257 and that genome size and RCV were positively correlated (Spearman rank correlation, $\rho = 0.89$, $P <$
258 0.001). Consistently, RCVs in the "large" selfed line were significantly higher than in the "small"
259 selfed line (Fig. 6c; Mann Whitney U-Test, $W = 88$, $P < 0.001$). Our model predicted the same
260 curvilinear relationship between genome size and RCV as we observed in *B. asplanchnoidis*, assuming
261 either 20Mbp or 34 Mbp elements (Supplementary Fig. 11). In particular, the prediction based on 20
262 Mbp elements seemed to match the majority of clones of the OHJ population. Altogether, the
263 positive correlation of RCV with genome size as well as the congruence of the model predictions and
264 the natural OHJ-population suggest that independently segregating elements are largely responsible
265 for genome size variation in *B. asplanchnoidis*.

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267

268

Discussion

Here we report a case of within-species genome size variation in the rotifer *B. asplanchnoidis* and show that even individuals *within* a natural population can differ by 1.33-fold, exhibiting genome sizes ranging from 414 to 552 Mbp (barring one extreme outlier at 792 Mbp). Moreover, artificial selection for large genome size can extend this range to 1.55-fold (640 Mbp) within just two generations. To our knowledge, these ranges represent some of the most pronounced cases of intraspecific variation in animals and plants (Supplementary Table 4).

Independently segregating elements are responsible for genome size variation

Within-population variation of genome size in *B. asplanchnoidis* is mainly mediated by multiple, large genomic elements that segregate independently during meiosis and can reach up to 34 Mbp in size. These elements are present in variable numbers in clones of the natural population. A very simple model, requiring as parameters only a minimum genome size (for individuals free of elements) and a vector describing size and number of all elements within a clone, can explain most observations in our data. This model is consistent with high heritability of genome size and the strong response to selection, while it accounts for the fact that we could not select below a minimum genome size. It could reproduce virtually all observed meiotic segregation patterns ("male peaks"), and it is consistent with the increase of male segregational variation (RCV) with genome size that we observe in the OHJ population (Fig. 6b). Finally, this model can explain the unexpectedly high genome size variation in a selfed line derived from a clone with large genome size.

On a chromosome level, independently segregating elements might resemble supernumerary chromosomes (B-chromosomes) that do not pair during meiosis. This is perhaps the most plausible explanation for the selfed line, which apparently accumulated up to six identical elements derived from an ancestor with three elements. If true, these B-chromosomes would be unusually large in their relative size, since one 34Mbp element corresponds to 8.2% of the minimum diploid base genome. B-chromosomes are usually much smaller than normal chromosomes (34), but may sometimes reach 3-5% of the diploid genome size (21, 34). A second possible mechanism is that the extra DNA might be integrated into the normal set of chromosomes, thus resulting in homeologous chromosomes of different length (e.g., 35). Accordingly, simple patterns of two or three male peaks would be caused by one or two elements located on different chromosomes, being present only once per chromosome pair. The two mechanisms are not mutually exclusive. For example in maize, B-chromosomes and "heterochromatic knobs" can contribute to intraspecific genome size variation (17). Unfortunately and despite much effort, we could not obtain reliable information on the

302 karyotypes of *B. asplanchnoidis*, likely because cell divisions in this organism are limited to a short
303 period when the embryo is encapsulated in a robust eggshell. However, we believe that genomic
304 analyses allow independent and possibly much deeper insights into the mechanistic basis of these
305 elements.

306 Our study allows precise predictions about patterns of coverage variation in genomic regions
307 that underlie these independently segregating elements. Indeed, preliminary comparisons among
308 clones of the selfed lines confirm that large genomic regions (spanning multiple Mbp in size) are
309 entirely missing in a clone with the minimal genome size (~414 Mbp), and that the same regions have
310 high and variable coverage in clones with larger genome sizes, at coverage ratios consistent with the
311 number of independently segregating elements (Blommaert et al. unpublished). Also recently, we
312 have compared the genome architecture of *B. asplanchnoidis* to that of other species of the *B.*
313 *plicatilis* species complex and found that transposable element abundance rather than an
314 allopolyploid origin explains the large genome size of *B. asplanchnoidis* (36). Even though *B.*
315 *asplanchnoidis* has a relatively small genome compared to other metazoans, it has a strikingly high
316 repetitive element content of 44%.

317

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

319 Previous studies have often assumed that genome size is a quantitative trait (QT) under
320 complete genetic control (24, 25), thus being determined by a large number of loci with each having
321 either positive or negative effects. Importantly, the QT model is oriented towards the mean genome
322 size of a population. Superficially, the high heritability of genome size and the strong response to
323 selection in *B. asplanchnoidis* support the QT model. However, we argue that the minimum genome
324 size, rather than the mean should be the point of reference when studying genome size variation on
325 a population level. Accordingly, the QT model refers to the special case, where all members of the
326 population have an elevated genome size. It is worth mentioning that the minimum genome size that
327 we defined for our species was not obvious from the distribution of the natural population (Fig. 1a),
328 but could only be established by experimental manipulation. Interestingly, previous reports of
329 intraspecific genome size variation in other organisms also report positively skewed distributions (22,
330 35), which could be an indication of a minimum genome size.

331 Our present model is strongly oriented towards a mechanism that involves completely
332 independent segregation of extra DNA. However, mechanisms where extra genomic material is
333 located on normal chromosomes can also contribute to genome size variation. Accounting for the
334 latter would require constraints regarding segregation patterns of extra DNA, particularly if these

335 elements are located on the same chromosome. In addition, extra genomic material may become
336 fixed in a population once all members carry the same extra DNA element on both chromosomes.
337 Thus, a more general model would incorporate both mechanisms, integration to normal
338 chromosomes and supernumerary chromosomes, and perhaps even an exchange between both
339 pools (37-40). In such a model, genome size could be highly dynamic on short time scales, due to
340 mechanisms that involve independent segregation, while fixation/loss of extra genomic material on
341 the normal set of chromosomes could explain the long-term changes in genome size across
342 populations or species. Overall, this model does not even contradict long-term gradual (Brownian
343 motion) changes in genome size, while it adds a population-level perspective to those taxa that seem
344 to have undergone saltations in genome size over macroevolutionary time scales (e.g., (41))

345

346 Conclusions

347 We have shown that within-population genome size variation in the rotifer *B. asplanchnoidis* is
348 mediated by relatively large, independently segregating genomic elements. Our data on short-term
349 evolution and inbred line variation suggest that a model involving only two variables, a minimum
350 genome size and a vector specifying the number/size of elements that increase genome size, is
351 sufficient for describing most aspects of genome size dynamics in this population. Collectively, our
352 study closes an important gap in our knowledge of how intraspecific genome size variation is
353 maintained in populations. This has general implications for identifying the evolutionary forces that
354 are responsible for the immense variation of genome sizes seen across eukaryotes. Most notably, it
355 should allow disentangling whether larger genome size can be beneficial, or whether it is always
356 slightly deleterious. In the future, it will also be interesting to elucidate in more detail the
357 mechanisms behind these independently segregating elements, for example their underlying
358 genomic architecture, or how they interact with the “non-dispensable” parts of the genome.

359

360

361 ## Methods

362 Resting eggs of rotifers were collected in the field from Obere Halbjochlacke (OHJ, N 47°47‘11“,E
363 16°50‘31“) and from Runde Lacke (N 47°47‘08“, E 16°47‘34“), two small alkaline playa lakes in
364 Burgenland (Austria) in 2011. Resting eggs from Lake Nakuru (Kenya) and the two Mongolian clones
365 were obtained from colleagues and have been previously described in detail (26, 31). All rotifers
366 were cultured as clones, consisting of the asexual descendants of the female that initially hatched
367 from a resting egg (for details, see Supplementary methods).

368 Genomesize measurements were performed with flow cytometry using a detergent-trypsin
369 method and propidium iodide (PI) staining according to (26) with minor modifications (for details, see
370 Supplementary methods). As an internal standard of known genome size, we used the fruit fly,
371 *Drosophila melanogaster* (strain ISO-1, diploid nuclear DNA content: 0.35 pg; (42)).

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

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

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

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

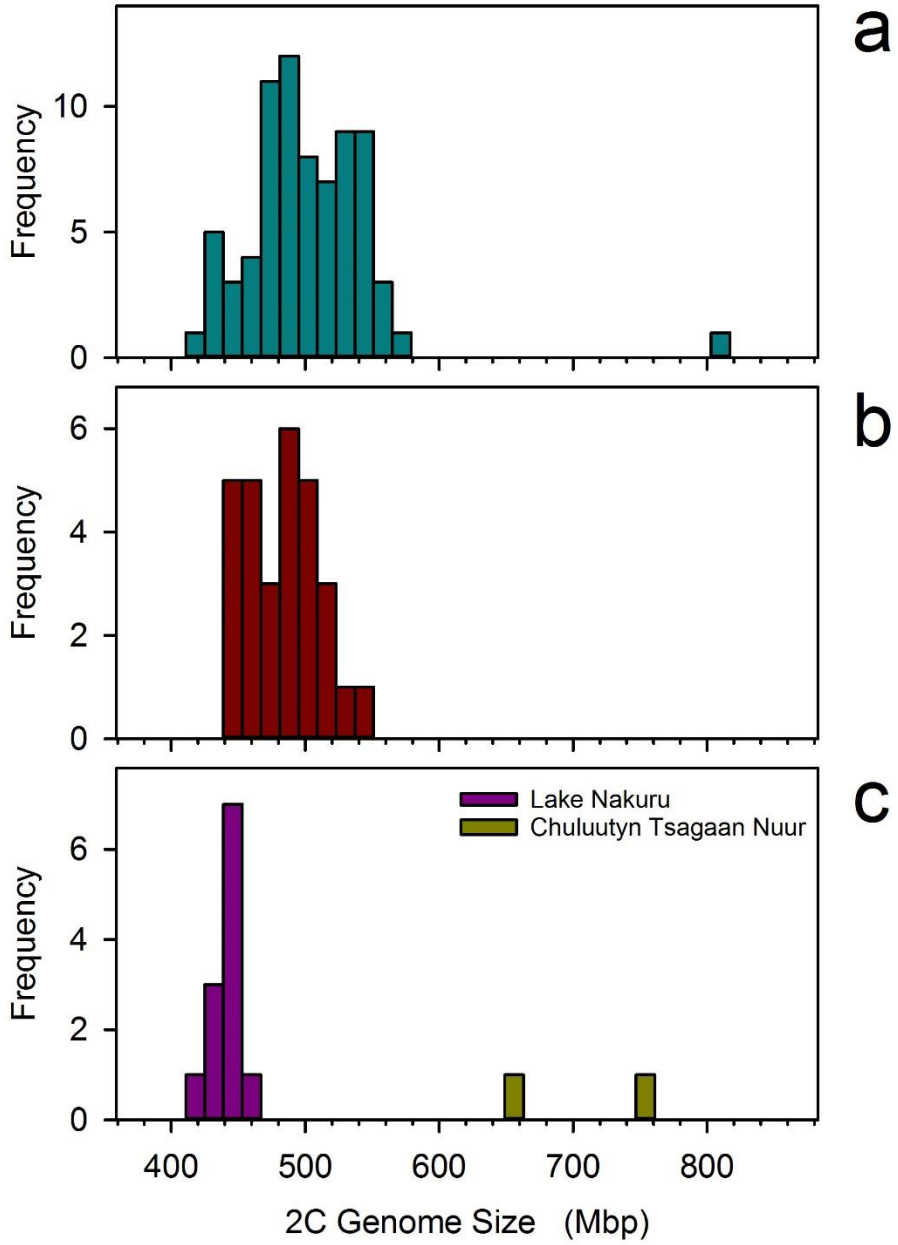
407 We performed two types of analyses depending on the quality our male peak data. First, in
408 clones that showed multiple discrete male peaks, we counted and sized these peaks (relative to the
409 female peak). We also determined the area under each peak, as a measure for the frequency of each
410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

412 $$RCV = CV_{allMP}/CV_{FP}$$

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

418 ## Figures and figure legends

Fig. 1



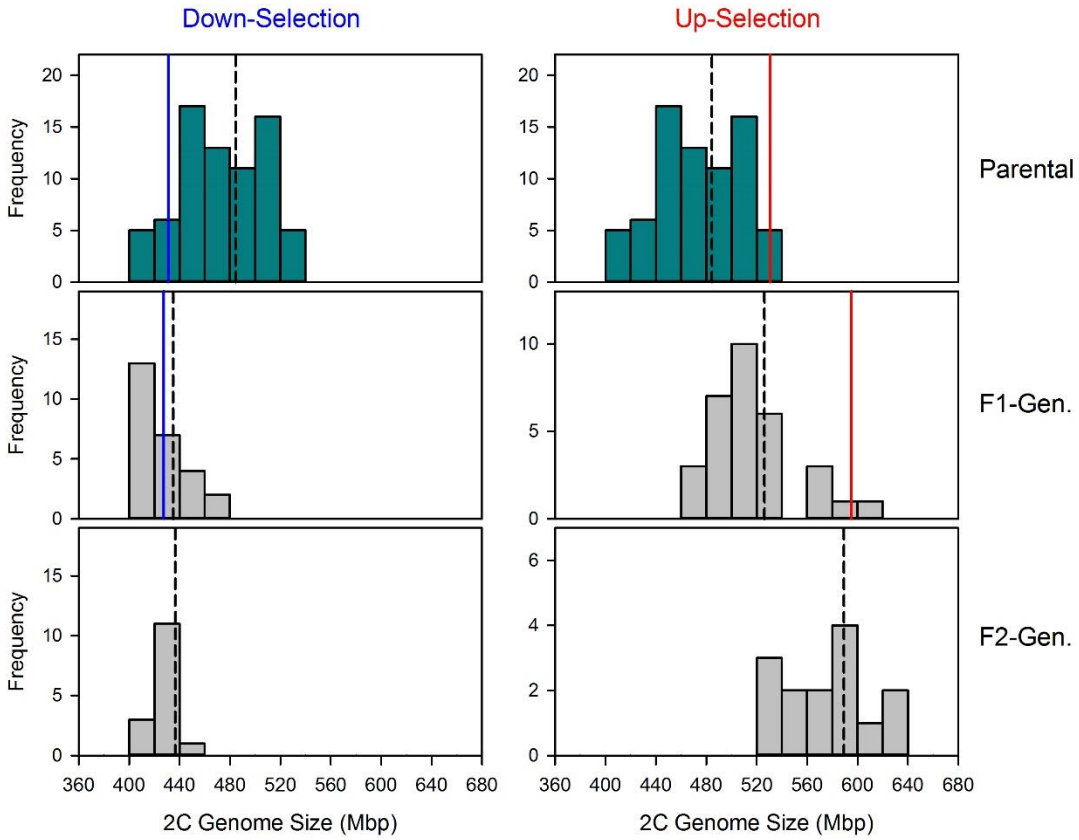
419

420 **Fig. 1** Genome size variation in four natural *B. asplanchnoidis* populations. **a** Obere Halbjochlacke
421 (Austria). **b** Runde Lacke (Austria). **c** Lake Nakuru (Kenya) and Chuluutyn Tsagaan Nuur (Mongolia).
422 Genome size was highly variable in the two Austrian populations (genome sizes ranging from 414 to
423 792 Mbp), whereas clones isolated from Lake Nakuru sediments differed little from each other (426 ±
424 5.1 Mbp, mean and standard deviation). The two clones from the Mongolian site had distinct and
425 relatively large genome sizes of 652 and 732 Mbp (Data from (31)).

426

427

Fig. 2



428

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

434

435

Fig. 3

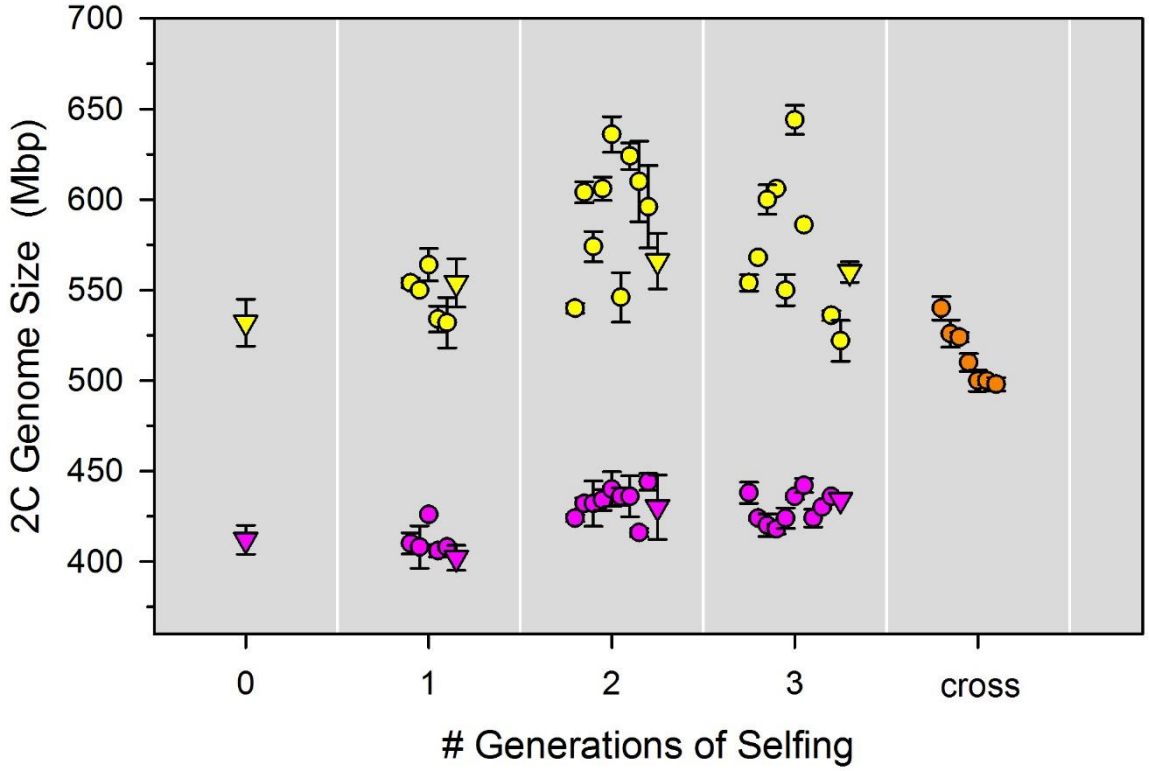


Fig. 3 Genome size variation in two selfed lines and their inter-line cross. Two selfed lines were established from two natural rotifer clones, one with large genome size (524 Mbp, yellow) and one with small genome size (414 Mbp, purple), and were propagated for three sexual generations. Clones used 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

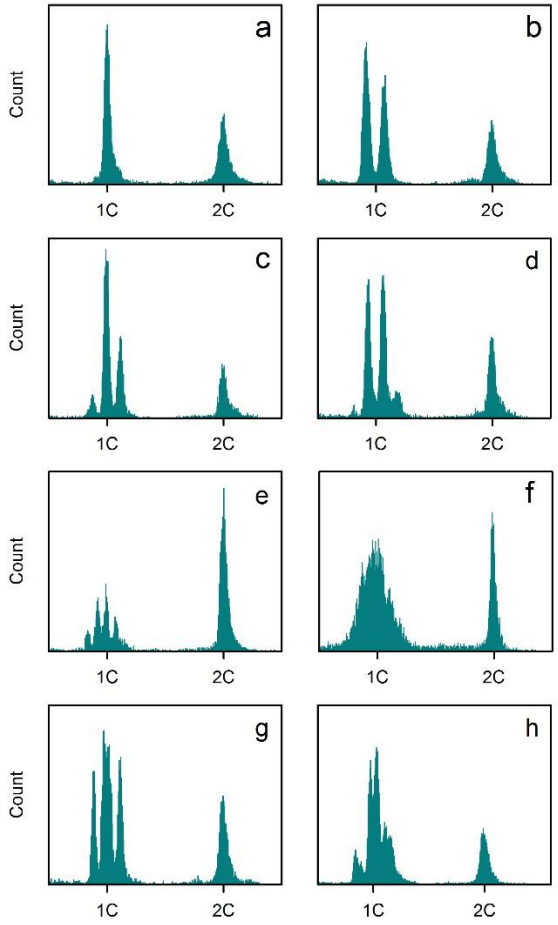


Fig. 4 Representative examples of genome size variation in haploid males. Each histogram corresponds to a different clone. X-axis corresponds to the YL2-A fluorescence on a linear scale. Only the section surrounding the 1C and 2C values is shown. The 1C value was set at 0.5x the median fluorescence of 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

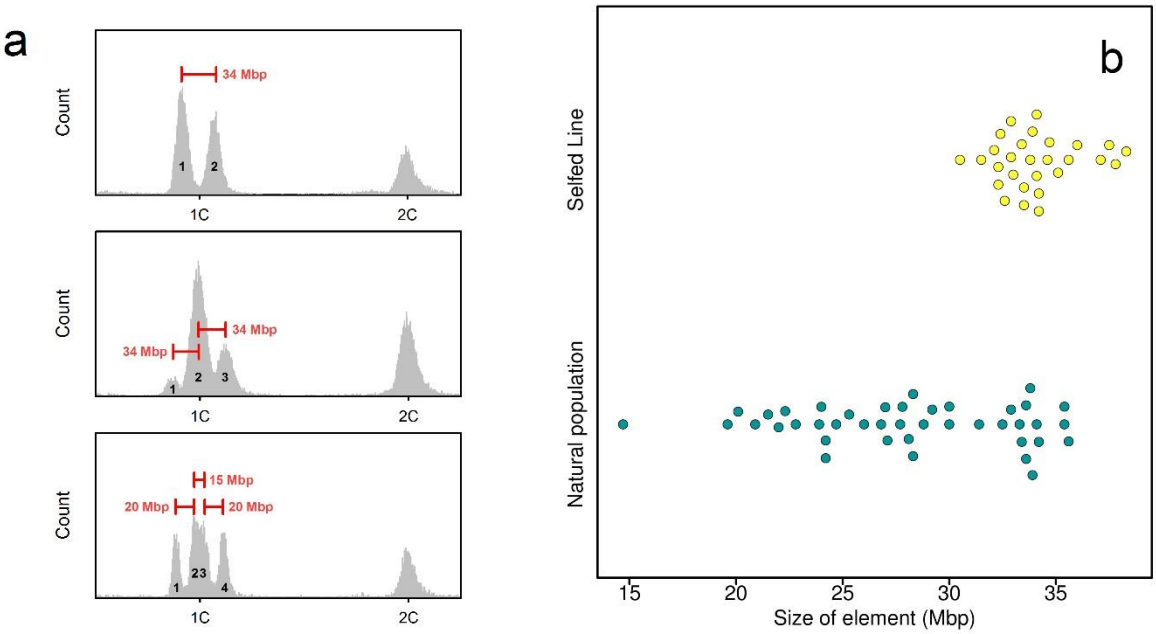


Fig. 5 Sizing of genomic elements responsible for male genome size variation. **a** Three examples of size inference, based on the distance between two male peaks (MPs) and knowledge of diploid genome size. Top: Example of a clone with two MPs, which can be explained by segregation of one ~34Mbp element. 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

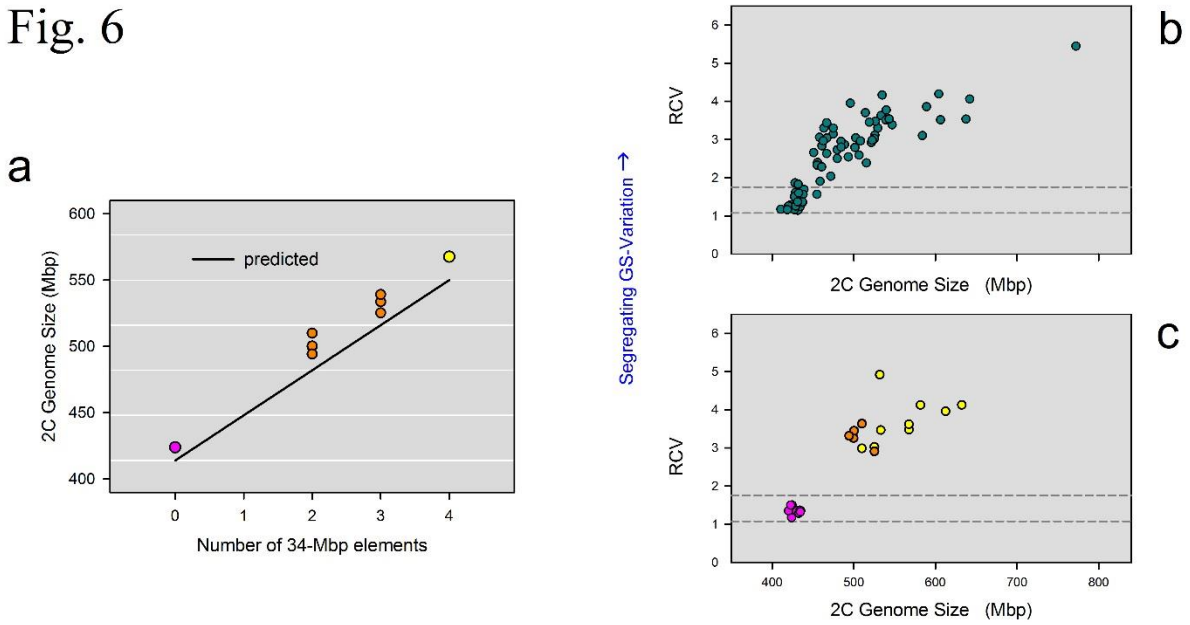


Fig. 6 Mechanistic explanations of intraspecific genome size variation. ***a*** Prediction of genome size based on the size and number of independently segregating elements, assuming a minimum genome size of 414 Mbp (i.e., free of elements). This figure shows seven clones of the inbred line cross (orange), 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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Summary of all supplementary information

Supplementary information

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

Supplementary file

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

Acknowledgements

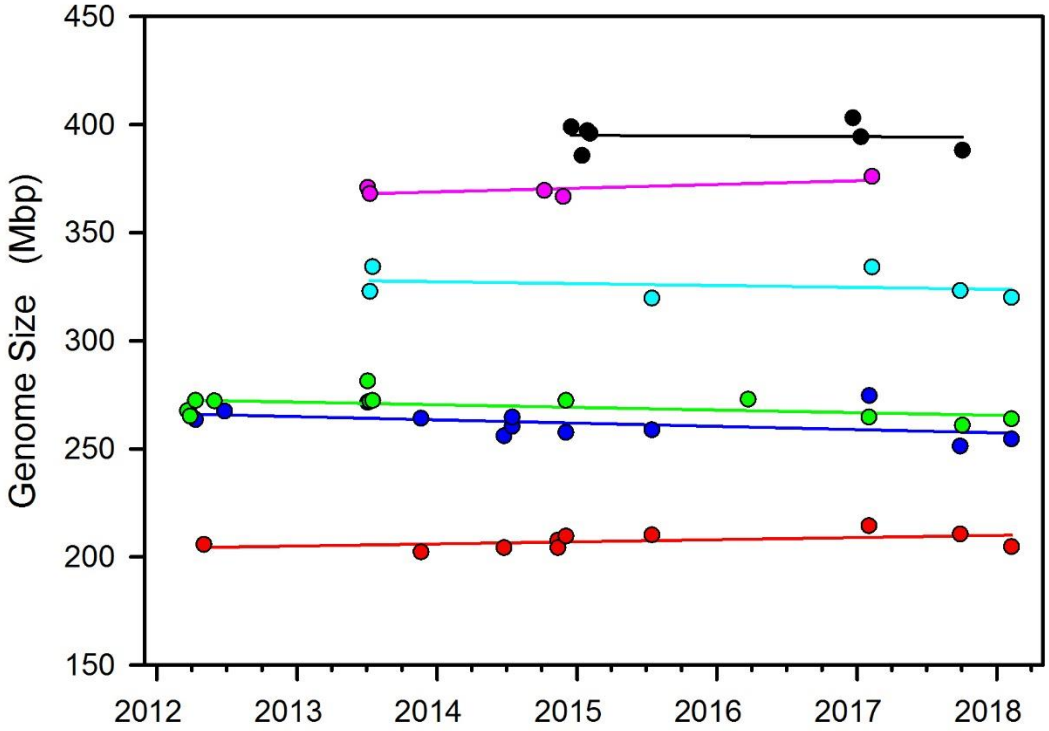
We thank Alois Herzig for providing access to the sampling sites and help during sampling of Obere Halbjochlacke and Runde Lacke. Sediments from Lake Nakuru were kindly supplied by Steve Omondi and Michael Schagerl. Julie Blommaert helped in preparation of media in some of the experiments. We thank David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

Authors' contributions

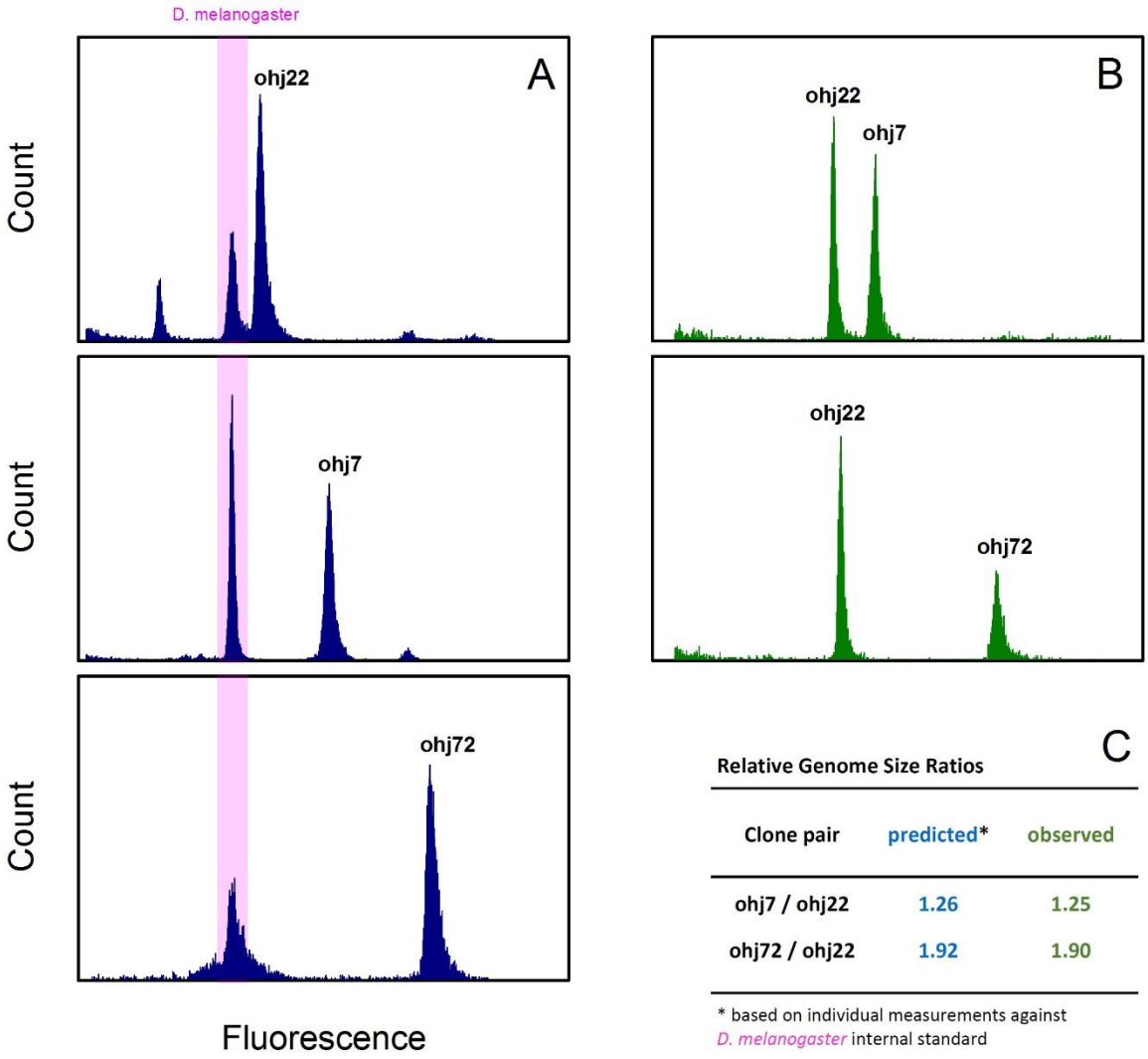
CPS conceived the study, designed and participated in all experiments. MP and PS contributed the flow-cytometry analysis. SR contributed the line crosses and the selection experiment. AH and MP did most of the culturing work and prepared rotifer biomass for flow cytometry. CPS analysed the data and drafted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

Supplementary Information

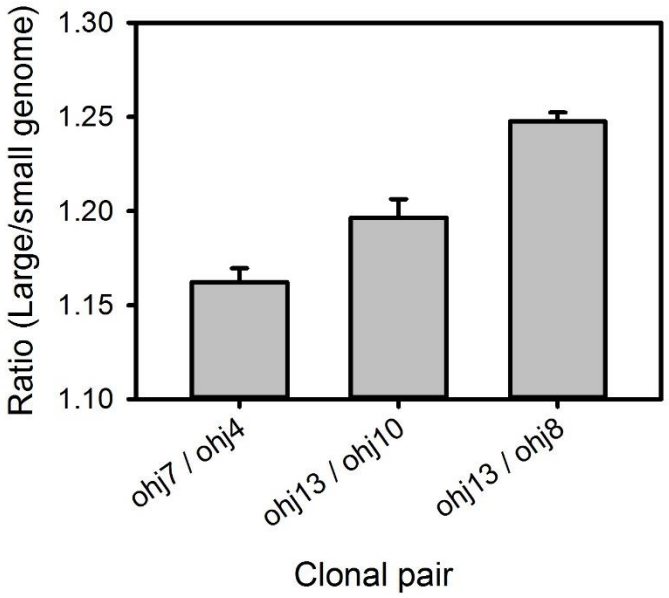
Supplementary Figures



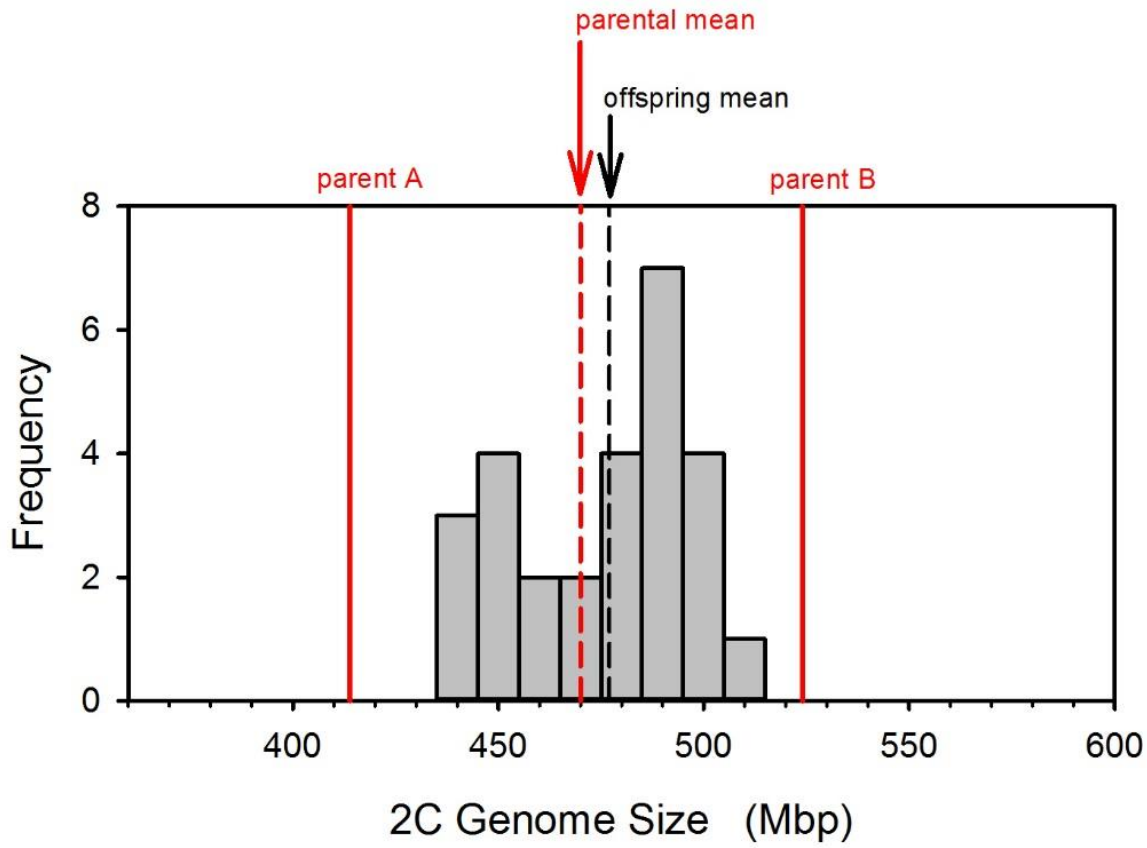
Supplementary figure 1. Long-term constancy of genome size during clonal propagation. Time courses of genome size for six *B. asplanchnoidis* clones over a period of > five years (i.e., approximately 600 asexual generations). Colours denote different clones: *ohj22* (red), *ohj7* (blue), *ohj13* (green),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



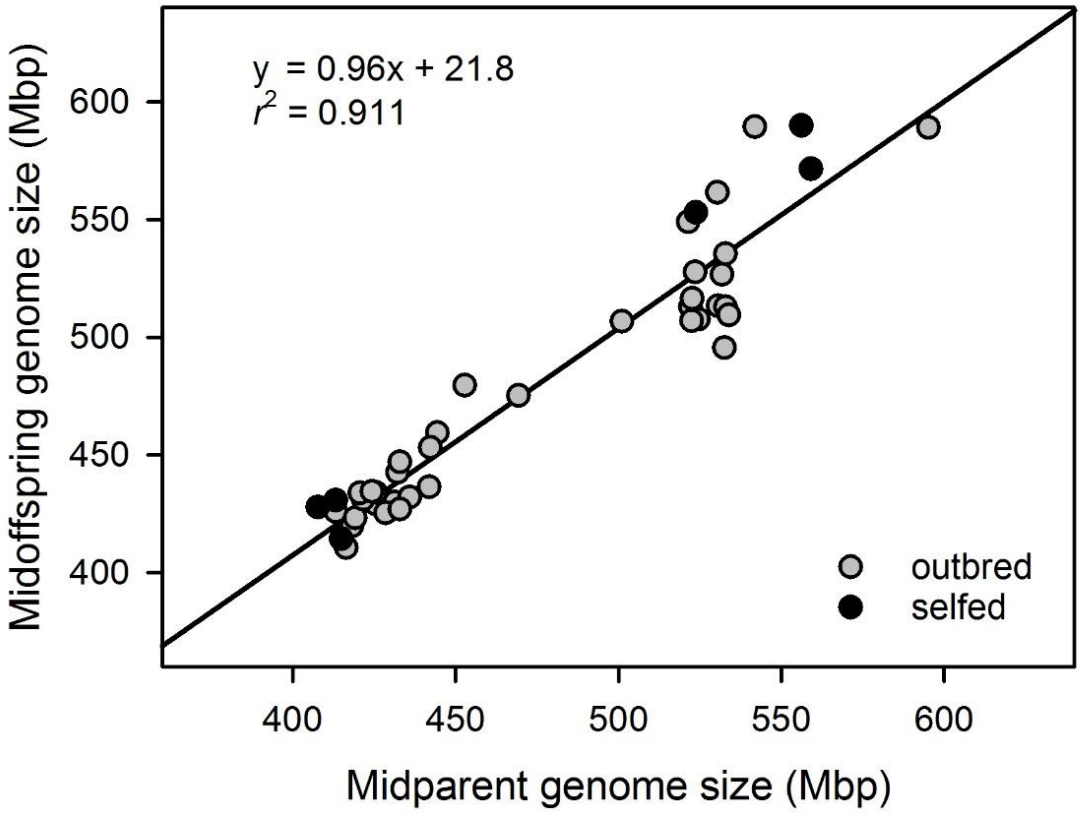
Supplementary figure 2. Comparison of two independent methods for measuring intraspecific genome size variation. a Three clones of different genome size were co-prepared with an internal standard of known genome size (*D. melanogaster*, strain ISO 1, 352 Mbp). **b** Pairs of the same three clones were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



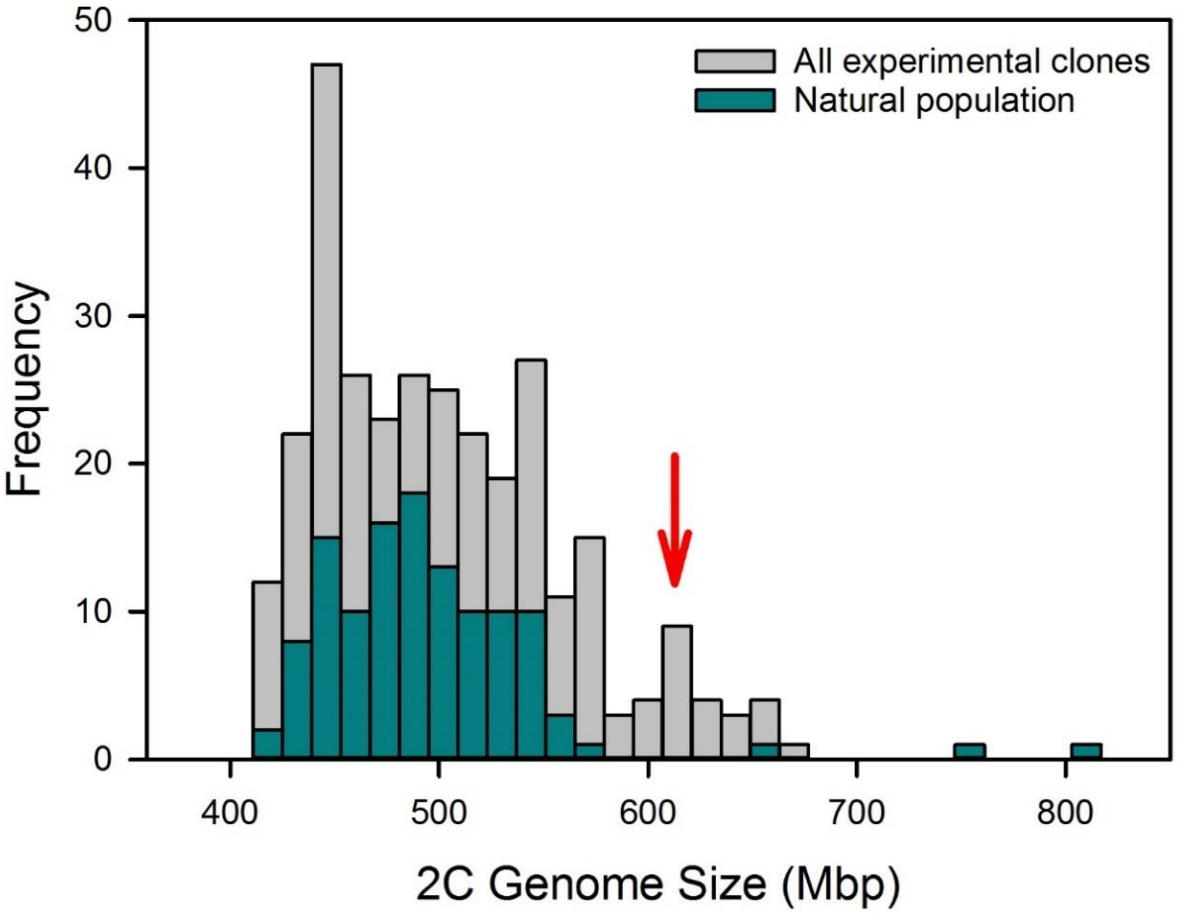
Supplementary figure 3. Genome size differences among selected pairs of clones in the OHJ-population. Error bars are standard deviations based on 3-4 replicates (measured on different dates). In each replicate the clonal pairs were co-prepared and stained in the same tube. Kruskal-Wallis test: *H*= 8.95,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



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

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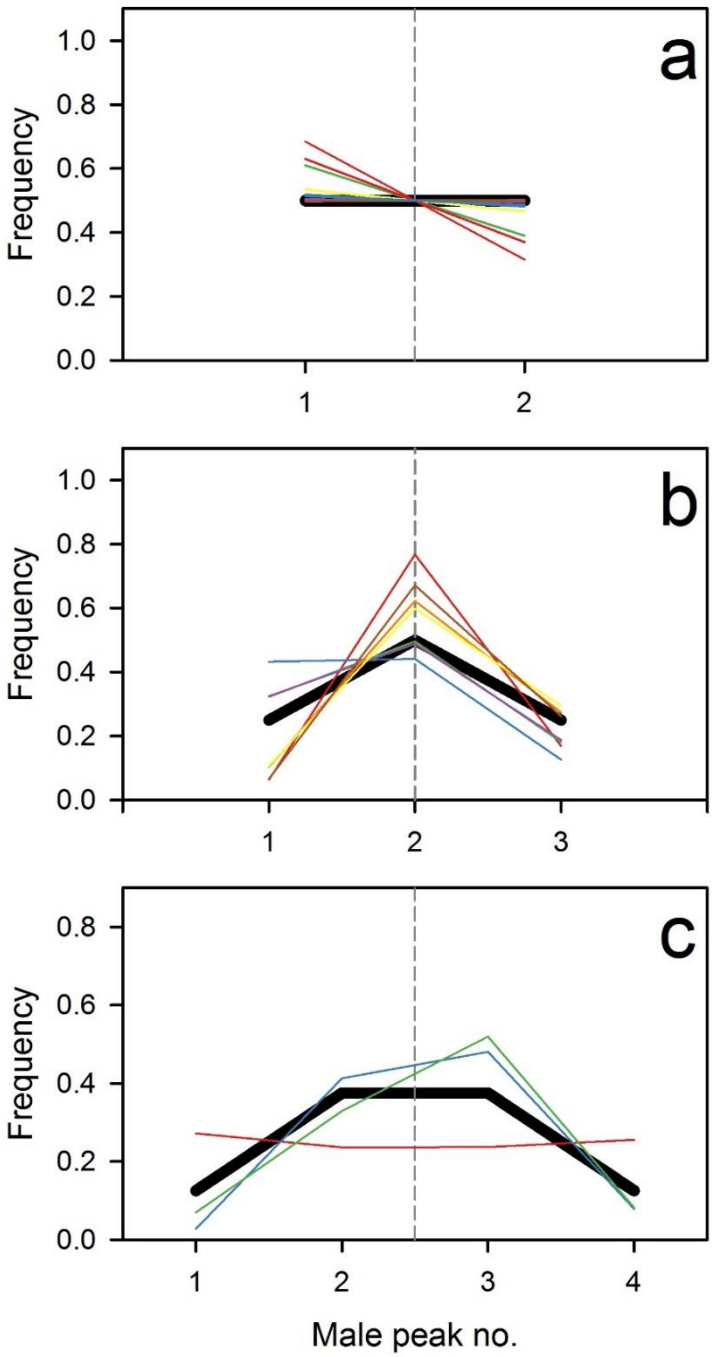

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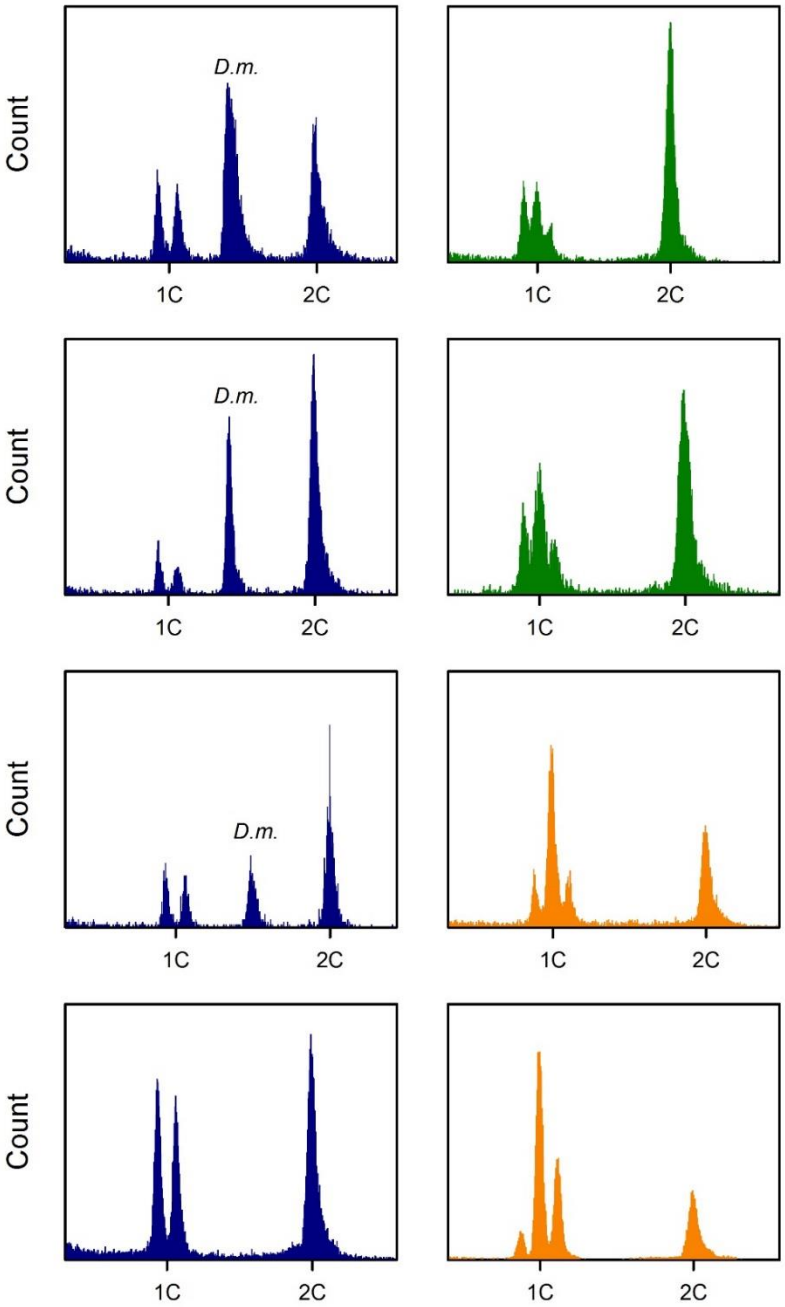
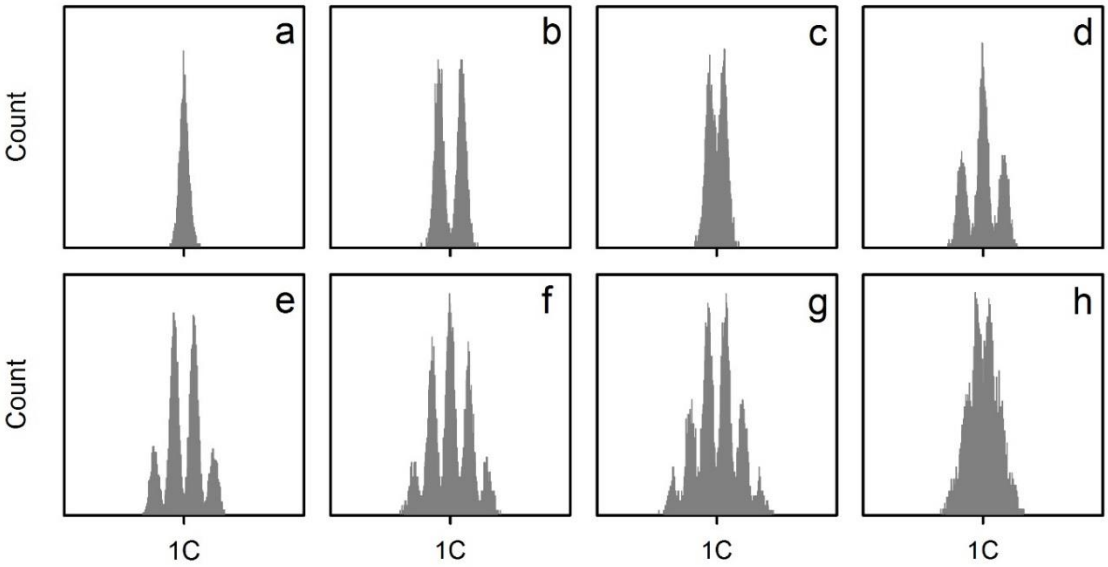
659 **Supplementary figure 7. Relative frequency of the different male genome sizes. a** two malepeaks;
660 prediction (thick line) based on one independently segregating element **b** three malepeaks;
661 prediction based on two equally sized elements, **c** four male peaks; prediction based on three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



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



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674 **Supplementary figure 9. Model simulations of "male peak" (MP) patterns with only one type of**
675 **independently segregating element. a** No element; **b** one element of 34 Mbp size; **c** one element of
676 20 Mbp; **d** two elements of 34 Mbp; **e** three elements of 34 Mbp; **f** four elements of 34 Mbp; **g** five
677 elements of 34 Mbp; **h** five elements of 20 Mbp. The model-parameter 'measurement precision' was
678 set to 2.7% (i.e., the coefficient of variance) in all cases.

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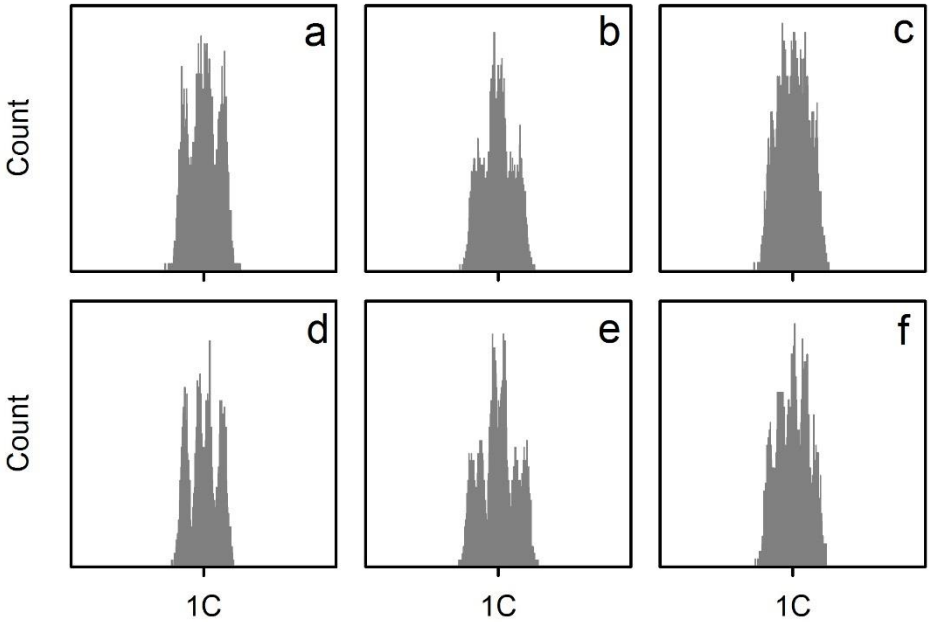

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683 **Supplementary figure 10. Model simulations of "male peak" (MP) patterns with different types of**
684 **independently segregating elements. a** two elements with 20 and 34Mbp; **b** three elements with 15,
685 34, and 34 Mbp; **c** three elements with 15, 20, and 34 Mbp. The model-parameter measurement
686 precision was set to 2.7% (i.e., the coefficient of variance). **d-f** are identical to **a-c**, except that
687 measurement precision was set to 2.0%.

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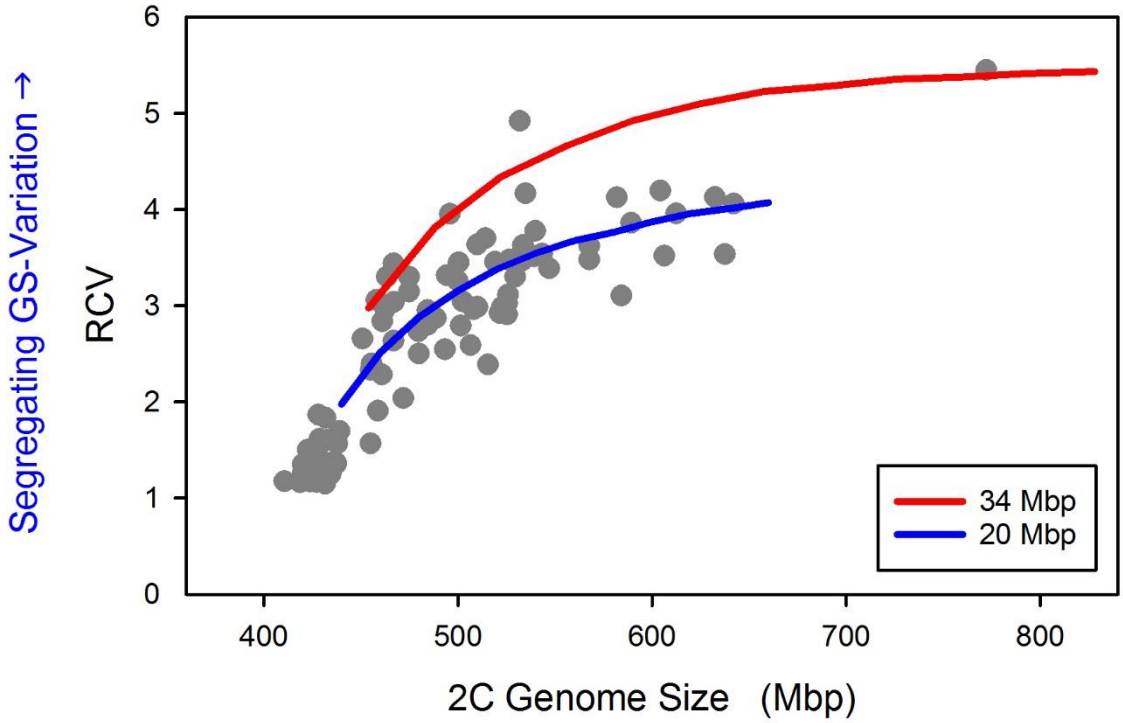

Supplementary figure 11. Model simulations of the relationship of RCV and genome size. RCV ("relative coefficient of variation") was calculated by dividing the CV of all combined male genome sizes (in a clone) by the CV of the female genome size. Red line shows model predictions for genomes with multiple 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)

701 ## Supplementary Tables

702

703 **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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710 ## Supplementary Methods

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712 ### Rotifer cultures

713 Rotifers were cultured in F/2 medium (43) at 16 ppt salinity and with *Tetraselmis suecica* algae as
714 food source (500–1000 cells μl^{-1}). Continuous illumination was provided with daylight LED lamps
715 (SunStrip, Econlux) at 30–40 µmol quanta m^{-2} s^{-1} for rotifers, and 200 µmol quanta m^{-2} s^{-1} for algae.
716 During the experiments, rotifers were cultured at 23°C. Stock cultures were kept either at 18 °C, re-
717 inoculated once per week by transferring 20 asexual females to 20ml fresh culture medium, or they
718 were kept for long-term storage at 9°C, replacing approximately 80% of the medium with fresh food
719 suspension every 4 weeks.

720

721 ### Genome size measurements

722 To determine the genome size of rotifer clones, we used flow cytometry and propidium iodide
723 staining of nuclei. Rotifer clones were grown in 250-1000 ml flasks until their population size reached
724 several hundreds to a few thousand individuals. On the day before preparation, rotifers were
725 harvested with 60 µm sieves, and starved overnight in 11 ppt 0.2µm-filtered medium, followed by
726 two washes with filtered medium on the next day. For each sample, we isolated 350 females and
727 subjected them to the flow cytometry protocol. Briefly, rotifers were homogenized in a citrate buffer
728 (3.4 mM Trisodium citrate dihydrate, Nonidet P40 at 0.1% v/v, 1.5 mM Sperminetetrahydrochloride,
729 0.5 mM Trishydroxymethylaminomethane, pH 7.6), and were then transferred to 750 µl stock
730 solution in a 1 ml Dounce tissue homogenizer. Rotifers were homogenized on ice with 20 strokes
731 using the "tight" pestle of the homogenizer. As internal standard of known genome size we used the
732 fruit fly, *Drosophila melanogaster* (strain ISO-1, nuclear DNA content: 0.35 pg, (42)). After adding two
733 female *Drosophila* heads, the sample was further homogenized with ten strokes. Large debris was
734 removed by filtration through a 40 µm mesh nylon sieve. After addition of 100 µl of 0.021% Trypsin
735 (dissolved in stock solution) the sample was incubated for exactly 10 min at 37°C. To prevent further
736 degradation, 75µl of 0.25% trypsin inhibitor was added (this solution also included 0.05% RNAse A)
737 and the samples were incubated for another 10 min at 37°C. Finally, samples were stained with
738 propidium iodide at a concentration of 50 µg/ml. Stained samples were kept overnight on ice in the
739 dark. Flow cytometric analysis was performed on the next day on an Attune NxT® acoustic focusing
740 cytometer (Thermo Fisher) with an excitation wavelength of 561 nm and a custom-made 590–
741 650 nm bandpass filter (yellow, YL-2) for detection of propidium iodide fluorescence. Flow cytometric
742 data were analyzed using FlowJo software version 10.0.7r2 (FlowJo LLC). To exclude doublets (i.e.,
743 nuclei that pass the detector too close together, thus being recorded as a single "event") we

744 employed YL2-A *vs*. YL2-H gating. Coefficients of variance (CVs) of individual peaks typically ranged
745 between 1.5% and 4% for both *Drosophila* and rotifers. Very few measurements had CVs higher than
746 5%, and those replicates were discarded. Conversion from picograms DNA to base pairs were made
747 with the factor: 1 pg =978 Mbp (42). In most cases, we obtained at least three replicate
748 measurements for a clone, usually on different days and sometimes with several weeks or months
749 apart.

750

751 ## Crossings between rotifer clones

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

764

765 ## Theoretical model of independently segregating elements

766 We conceptualized a theoretical model to summarize our finding of independently segregating
767 genomic elements. The main purpose of this model was to test the hypothesis that these genomic
768 elements are *sufficient* as the mechanism of genome size variation. In particular, we examine
769 whether such a model is consistent with our observations of genome size variations in males from
770 clones of different diploid genome size. The basic input parameters and variables of the model are:
771 (i) an assumed minimum diploid genome size of 414 Mbp (for a clone that contains no additional
772 genomic elements), and (ii) a vector describing the size and number of individual elements in a clone
773 (e.g., [34 34 20] for two 34 Mbp and one 20 Mbp element, respectively). The output variable of the
774 model was the predicted diploid genome size of females, calculated as minimum genome size + the
775 summed contribution of all elements. The second output variable was the genome size distribution
776 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;
```