Genomic Convergence toward Diploidy in *Saccharomyces cerevisiae*

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Genome size, a fundamental aspect of any organism, is subject to a variety of mutational and selection pressures. We investigated genome size evolution in haploid, diploid, and tetraploid initially isogenic lines of the yeast *Saccharomyces cerevisiae*. Over the course of ~1,800 generations of mitotic division, we observed convergence toward diploid DNA content in all replicate lines. This convergence was observed in both unstressful and stressful environments, although the rate of convergence was dependent on initial ploidy and evolutionary environment. Comparative genomic hybridization with microarrays revealed nearly euploid DNA content by the end of the experiment. As the vegetative life cycle of *S. cerevisiae* is predominantly diploid, this experiment provides evidence that genome size evolution is constrained, with selection favouring the genomic content typical of the yeast's evolutionary past.

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Introduction

Organisms vary tremendously in genome size [1,2] yet the key evolutionary forces acting to shape genome size in any particular organism remain unclear. Genome size is subject to small-scale changes (gene insertions or deletions) as well as large-scale ploidy differences (changes in the number of full chromosome sets). Genome size is known to influence a variety of phenotypes, including cell size [3], generation time [4], ecological tolerances [5], and reproductive traits [6]. Gene copy number is also thought to affect long-term rates of evolution, by altering the available number of mutations [7] and the efficacy of selection [6,8,9]. Using the budding yeast *Saccharomyces cerevisiae* as a model system, experimental evolution studies have confirmed the influence of genome size on long-term rates of evolution [7,10,11]. Genomic composition can, in turn, evolve over the course of such experiments. Recent experiments provide strong evidence that genomic changes, including insertions, deletions, and translocations, contribute to adaptation to novel environments in both *Escherichia coli* [12] and *S. cerevisiae* [13].

To investigate the evolutionary importance of genome size, we evolved initially isogenic haploid, diploid, and tetraploid *S. cerevisiae* for 1,766 asexual generations in batch culture. Five replicate lines of each ploidy level were grown in two experimental environments: an unstressful medium consisting of standard lab YPD (yeast extract peptone dextrose), and a salt-stressed medium consisting of YPD and 0.6 M NaCl. Large-scale (ploidy level) changes in genome size throughout the timescale of the experiment were identified using flow cytometry, while relative changes in gene copy number, including aneuploidies and indels, were identified using comparative genomic hybridization (CGH) of genomic DNA to microarrays at the final time point. Remarkably, we found convergent evolution among initially haploid and initially tetraploid lines toward diploidy, the predominant vegetative state of *S. cerevisiae* [14]. These results suggest that genome size is subject to evolutionary inertia, with selection opposing shifts in ploidy away from the historical level.

Results/Discussion

Evolution with respect to genome size was surprisingly consistent: all strains converged toward or remained diploid (Figure 1), the predominant vegetative state of *S. cerevisiae*. Diploid individuals appeared and rose to high frequency through all ten replicate haploid populations (25 colonies were sampled from each line at generation 1,766; only one out of 250 colonies was still haploid), in both unstressed (Figure 1A) and salt-stressed (Figure 1B) media. Similarly, all ten initially tetraploid lines decreased in genome size (unstressed medium, Figure 1E; salt-stressed medium, Figure 1F). Cells of approximately diploid DNA content were found in all 5×25 colonies sampled at generation 1,766 from the five unstressed medium lines and from 25 colonies sampled from one of the salt-stressed lines (line qs), while cells of approximately triploid DNA content were observed in the 4×25 colonies sampled from the remaining four salt-stressed lines. Considerable polymorphism for genome size was apparent at earlier time points in this experiment for both initially haploid and initially tetraploid lines (Figures 1 and S1). Diploid lines showed no large-scale changes, though smaller-scale fluctuations in genome size occurred throughout the time series in both unstressed (Figure 1C) and salt-stressed (Figure 1D) media. The pattern of convergence towards diploidy was confirmed in a second independent experiment (Figure S2).

To find out whether chromosomes were present in euploid

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Abbreviations: CGH, comparative genomic hybridization; MAT, mating type; YPD, yeast extract peptone dextrose

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Synopsis

Genome size is a fundamental aspect of all species and has the potential to influence a number of individual characteristics such as cell size, generation time, ecological tolerances, and reproductive traits. Although genome sizes range widely among species, the forces shaping the evolution of genome size are only poorly known. Here we provide the results of an ~1,800 generation evolution experiment using lines of the budding yeast *S. cerevisiae* with either one, two, or four copies of their genome (haploid, diploid and tetraploid, respectively). We found, surprisingly, that all haploid and tetraploid lines converged toward diploidy, the historical state of *S. cerevisiae,* by the end of the experiment. Further experiments suggest that entire sets of chromosomes were lost as genome size changed from tetraploid to diploid. Our results suggest that genome size is constrained by selection acting against changes from the historical genome size.

or aneuploid ratios in the evolved tetraploid lines, CGH was performed. Chromosomal content of the tetraploid cells was generally close to euploid, regardless of the samples compared. A euploid index was calculated from each array comparison where 1 indicates euploidy, while each chromosome deviating substantially (>10%) from the expected ratio reduces the index by 1/16. The euploid index was 0.891 between pairs of samples compared from early in the experiment (within the first 200 generations; Figure S3), 0.896 for samples compared from the end of the experiment (generation 1,766) to ancestral samples (generation 0, Figure S4), and 0.927 for two samples from the end of the experiment (Figure S5; estimates are likely to be biased downward; see Protocol S1). Although minor or partial aneuploidy may have been present in the initial lines, we did not detect any missing regions in the microarray analysis, indicating that no large-scale aneuploidy was fixed in the initial lines. Thus, while some aneuploidy was present at the end of our experiment, it typically involved few chromosomes (often Chromosome 9) and was not consistent with random chromosome loss. Interestingly, CGH also detected at least three indels (Figure 2A, Figure S6). Of particular interest was a duplication within one of the tetraploid lines reared in salt-stressed (Figure 2Ai, line qs), encompassing several genes on Chromosome 4 involved in sodium efflux (the P-type

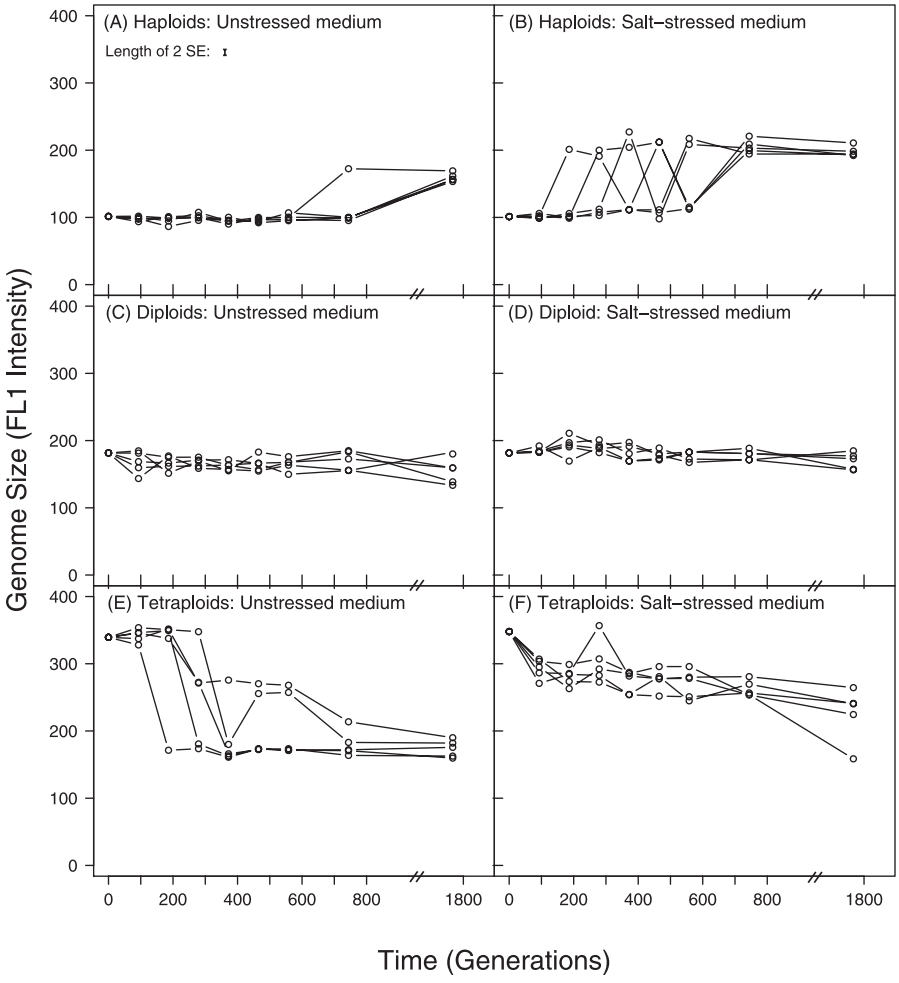

Figure 1. A Snapshot of Genome Size Change across 1,766 Generations of Batch Culture Evolution

Each data point is the mean of three FACScan measurements on a single colony sampled from a population. Apparent fluctuations are largely a result of genome size polymorphisms, leading to sampling fluctuations depending on which colony was randomly chosen (see Figure S1). The average standard error (shown in panel A) reflects measurement error. FL1 represents a linear scale of dye fluorescence as measured by flow cytometry. The five lines on each graph represent the five replicate lines evolved independently.

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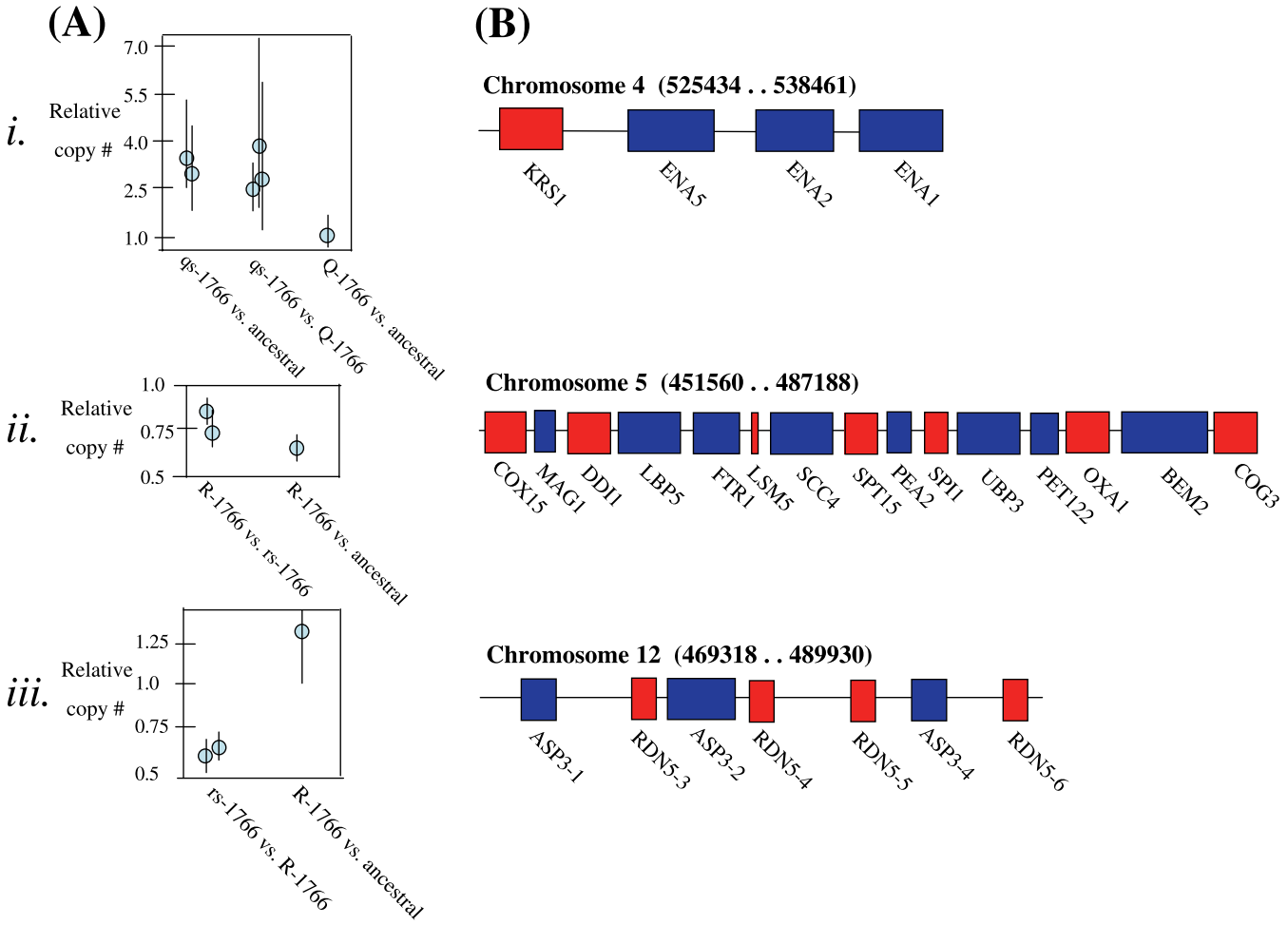

Figure 2. The Three Indels Identified by CGH Analysis of Ancestral (Generation 0) and Evolved (Generation 1,766) Tetraploid Lines

(i) An insertion of a ~13-kilobase fragment on Chromosome 4 in tetraploid salt line qs; (ii) a potential ~36-kilobase deletion of Chromosome 5 in tetraploid line R; (iii) a potential 20-kilobase deletion of Chromosome 12 in tetraploid salt line rs.

(A) Results of CGH, where each dot represents the mean (bars: 95% CI) relative copy number of all genes in the indel from a single array.

(B) Genes of known function (http://www.yeastgenome.org, 2 October 2005) affected by the indel (basepair range of genes involved are given in brackets). Each box is one ORF, where red indicates transcription on the Watson strand, and blue for genes transcribed on the Crick strand.

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ATPases, *ENA1* (YDR040C), *ENA2* (YDR039C), and *ENA5* (YDR038C)). Deletions within Chromosome 5 (Figure 2Aii) and Chromosome 12 (Figure 2Aiii) were also identified (Protocol S1).

The driving force behind the genomic size decrease in tetraploid lines could have been selection or mutational bias (due to deletions outnumbering insertions). To distinguish between these possibilities, we conducted a bottleneck experiment in unstressed medium starting from the same ancestral tetraploid culture. By reducing the population size every 48 h to a single cell by picking and streaking single random colonies, we limited the variability necessary for selection to act. Consequently, mutational biases and drift became the dominant evolutionary forces. Results were significantly different from the original experiment. After 572 generations (26 bottlenecks), diploids were present in only two of ten bottlenecked lines compared with five out of five lines evolved at large population sizes over this same time period in the original experiment (Fisher's exact test, $p = 0.007$; Figure S7). The average genome size observed in the primary experiment (181.48 FL1 intensity ± 7.87) was also significantly lower ($t_{11} = 4.697$, $p = 0.0003$) than the average genome size from the bottlenecked populations (286.06 ± 20.83). Therefore, selection on genome size is required to account for the rapid convergence toward diploidy observed in Figure 1.

Ploidy level and environment showed a significant interaction ($F_{2,24} = 3.595$, $p = 0.0431$) on the pattern of genome size evolution (Figure 3). Initially, haploid lines increased in genome size faster in salt-stressed medium ($t_8 = 3.729$, $p = 0.0058$), while initially tetraploid lines decreased in genome size slower in salt-stressed medium ($t_8 = -3.948$, $p = 0.0042$). Although the diploid strains showed only small-scale decreases in genome size, the rate of loss was also significantly more rapid in unstressed medium ($t_8 = -3.2715$, $p = 0.011$).

Because the lines used in this study lack a pheromone receptor required for mating and carry a mutation-preventing sporulation, syngamy and meiosis should not have occurred. To ensure that mate switching did not occur in the haploid lines (which might have allowed for sexual reproduction), PCR of the mating type (MAT) locus was performed. We found that only the MATa allele was present

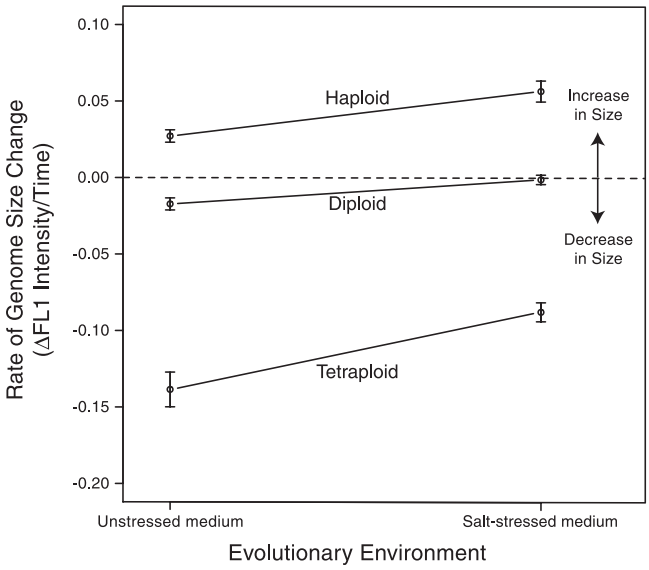

Figure 3. Rate of Genomic Size Change by Ploidy and Environment
Rate of change was calculated by fitting linear regression lines through timeseries data (Figure 1) for each individually evolved line. Each data point thus represents the mean ± SE of five slope measures. This figure shows that haploids increased in genome size faster in salt, while tetraploids decreased in genome size more slowly in salt.
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at this locus in all haploid-evolved lines (generation 1,766), arguing against mate switching. We also performed a sporulation assay using known protocols [15] on all lines at the end time point (1,766 generations). No spores were found in any experimental lines, though sporulation was observed in a positive control using a yeast strain known to sporulate [16]. Thus, it is unlikely that meiosis occurred during the course of the experiment. One plausible mechanism for mitotically occurring genome size change is that haploid individuals in both unstressed and salt-stressed media underwent endomitosis (chromosome replication not followed by division), creating diploid offspring with two copies of each haploid parental gene. The process by which tetraploids lost DNA remains unclear, but appears to follow roughly euploid shifts in chromosome content. The time frame over which we observed euploid shifts in DNA content from tetraploid to diploid was short (100–200 generations, Figure 1). For 32 chromosomes to be lost in rapid succession within such a short time frame, there would had to have been at least a 550% fitness gain each time a chromosome was lost (Protocol S1). As the initial growth rate of the tetraploid lines was only marginally reduced (by 5%–10% relative to haploids and diploids), it is highly unlikely that the transition to diploidy involved the independent appearance and selective spread of cells that lost one chromosome at a time. This argues for a concerted mutational process involving the loss of multiple chromosomes, generating mutant cells that are approximately euploid and that are selectively favoured (as demonstrated by the bottleneck experiment; Figure S5). Such rapid and concerted loss of multiple chromosomes has been observed in *Candida albicans* [17], a historically diploid yeast species closely related to *S. cerevisiae*.

Typically, models of ploidy evolution predict that either larger genomes or smaller genomes are favoured, depending on the environment, population size, and reproductive system of the organism [6,18]; intermediate ploidy levels are not generally expected in the absence of constraints. Yet in our study we find strong, repeated evidence for selection on diploidy, the intermediate ploidy level.

As *S. cerevisiae* is historically diploid, we conjecture that selection has acted over evolutionary time to optimize organismal function with two full sets of chromosomes. After a period of time at a particular ploidy level, an organism might become well adapted to the attendant cell size and gene expression patterns, reducing the fitness of ploidy mutants. For example, even isogenic *S. cerevisiae* strains of different ploidy levels exhibit altered gene expression patterns [19], which might select against lines with novel ploidy levels. We hypothesize that diploids potentially have a competitive advantage over haploids. Although no significant competitive differences were detected by a different study using the same strain [20], the power of this study was such that a small difference in competitive ability (i.e., 10%) could not have been detected. Consistent with our hypothesis, a study comparing haploid and diploid individuals of historically haploid *(Schizosaccharomyces pombe)* and diploid *(S. cerevisiae)* yeast found that evolutionary history, rather than environmental conditions, predicted individual competitive performance and growth rates (V. Perrot, personal communication). Whether convergence toward haploidy would be observed in historically haploid yeast remains to be seen and would allow us to distinguish between evolutionary inertia or historical constraint versus a generalized advantage of diploidy.

The rate of convergence toward diploidy was highest for haploids in salt-stressed medium and tetraploids in unstressed medium. Coupled with our finding that initially tetraploid lines in the salt-stressed medium have a significantly higher genome size at generation 1,766 relative to initially tetraploid lines grown in unstressed medium ($t_8 = 2.75$, $p = 0.025$), we conclude that a large genome size was slightly more favourable in the salt-stressed medium. This result suggests that the adaptive benefits of higher or lower genome size are affected by the ecological environment. Thus, while ploidy itself might be constrained by historical factors, the rate of adaptation with respect to genome size change is likely influenced by the environment.

Here we have shown that an intermediate ploidy is selectively favoured in two different environments. Our results suggest that evolutionary inertia might act to constrain genome size evolution, preventing shifts away from the ploidy level to which an organism has historically adapted.

Materials and Methods

Generation of lines. Haploid, diploid, and tetraploid lines of *S. cerevisiae* were initiated from culture frozen down by B. Mable [18] (strains BM1N, BM2N, and BM4N descended from haploid strain SM2185 kindly provided by A. Adams and S. Brower). These three lines were isogenic with haplotype *MATa-a1 ste6Δ8–694 ura3 leu2 his4 trp1 can1*. A deletion in the pheromone receptor locus *(ste6Δ8–694)* and a mutation in the MAT locus (*MATa,* mutation *a1*) should prevent mating [21] and sporulation [22], respectively, and eliminate any potential pleiotropic effects of the MAT locus on relative fitness [23].

Batch culture evolution. Ancestral ploidy lines were initiated by streaking frozen stock (BM1N, BM2N, and BM4N) onto YPD plates, picking off a single colony after 48 h, and culturing for 24 h in the appropriate (unstressed or salt-stressed) liquid medium. Unstressed

medium was YPD (Difco, Sparks, Maryland, United States). The salt-stressed medium was unstressed medium plus 0.6 M NaCl, which reduced initial growth rates by 35%. Culture was then frozen down at −80 °C in 15% dimethyl sulfoxide (DMSO; Sigma, St. Louis, Missouri, United States) as the six time zero lines (three ploidy levels × two environments). 5 × 100 ul from each of these six initial tubes was then pipetted into 10 ml of appropriate medium and used to initiate five replicate lines. 266 daily (24 h ± 1 h) 1:100 transfers (100 μl culture into 10 ml medium) were conducted sequentially. As each transfer allowed ~6.64 mitotic divisions ($2^{6.64} = 101$) before the population returned to stationary phase, a total of ~1,766 (= 266 × 6.64) cell generations occurred per line regardless of environment. Cultures were continually shaken at 200 rpm and maintained at 30 °C overnight. Subsequent freezing was conducted every 2 wk (93 generations).

Genome size determination. Flow cytometry (FACScans) was used to determine relative ploidy of all lines at nine different time points: 0, 93, 186, 279, 372, 465, 558, 744, and 1,766 cell generations. The FACScan protocol [24] was modified as described by the Fred Hutchinson Cancer Research Center (http://www.fhcrc.org/science/labs/gottschling/yeast/facs.html). Cells from frozen culture were streaked to single colonies on YPD plates. A single colony was picked off and grown in YPD for 24 h. The only deviation in protocol was that the last pellet was resuspended in 980 μl of sodium citrate and 20 μl of 0.05 mM SYTOX Green dye. Cultures were kept at room temperature for a minimum of 3 h (but up to 24 h) to ensure dye uptake and then stored at 4 °C overnight.

30,000 cells from each culture were analyzed on a FACSCalibur (Becton-Dickinson Immunocytometry Systems, Palo Alto, California, United States). The FL1 detector was used for the acquisition of SYTOX Green fluorescence, where dye is taken up by the cells in a manner stoichiometric to the amount of DNA in the nucleus. The FlowJo (Tree Star, Ashland, Oregon, United States) cell cycle analysis function using the Watson pragmatic option was used to fit Gaussian curves to our data to determine the FL1 intensity corresponding to the G1 mean, which indicates the average unreplicated DNA content of each population of cells [25].

FACScans over all time points for the five replicate lines of each treatment (45 tubes) were performed on the same day. The entire protocol was replicated on three different days using cells from the same colonies; any variation reflects machine/treatment variation and not genetic variation within the cultures. A significant day effect was found, and the data were corrected by adjusting the replicate data collected on different days to have the same mean. The corrected data were used for subsequent analyses.

Microarrays. On a subset of the tetraploid lines, we used CGH of genomic DNA of microarrays to determine whether the evolved lines were euploid or aneuploid [26,27]. For each CGH, a colony was isolated from frozen stock and grown to stationary phase in liquid YPD. Genomic DNA was extracted from 8 ml of stationary phase culture using a standard yeast mini-prep DNA isolation procedure [28]. Genomic DNA (5 μg) was sonicated (3 × 10 s at 45% of 20 kHz) to obtain DNA fragments of roughly 100 basepairs to 10 kilobases and purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, California, United States). The two DNA samples to be compared were labeled with Cy3 or Cy5 using the Mirus Label IT Nucleic Acid Labeling Kit (Mirus, Madison, Wisconsin, United States), according to the manufacturer's protocol. We then co-hybridized the labeled genomic DNA to *S. cerevisiae* microarrays obtained from the University Health Network Microarray Centre (Toronto, Ontario, Canada). The hybridized slides were washed and scanned using ScanArray Express (PerkinElmer, Wellesley, California, United States) set to the yeast protocol. QuantArray (PerkinElmer) was used to quantify the relative fluorescence of Cy3 and Cy5 between the two samples of interest. Finally, GeneSpring (Agilent, Palo Alto, California, United States) was used to order the data according to chromosomal location.

CGH analysis. All fluorescence ratios were log-transformed prior to analysis and back-transformed for presentation. The average fluorescence ratio was first calculated for each chromosome to assess the degree of aneuploidy. A 99.8% confidence interval for the chromosomal average ratio was obtained by bootstrapping. Bootstrapping involved randomly sampling from the gene ratios observed within a particular chromosome with replacement, yielding a bootstrap dataset with the same number of data points as the original chromosome; 1,000 bootstrap datasets were obtained per chromosome. A 99.8% confidence interval was chosen to correct for multiple comparisons across the 16 chromosomes of *S. cerevisiae* (giving an overall alpha value per genome of $\alpha = 0.03$).

As the same concentration of DNA was hybridized to each microarray, a CGH analysis cannot assess relative differences in ploidy level between lines. Aneuploidy of a chromosome can be detected, however, as a departure from a fluorescence ratio of one in a CGH comparison of two otherwise euploid genomes. A decrease in copy number of a particular chromosome is expected to lead to a 0.5 ratio (in diploids), 0.67 (in triploids), or 0.75 ratio (in tetraploids) relative to the rest of the chromosomes. Conversely, an increase in copy number of a particular chromosome is expected to lead to a 1.5 ratio (in diploids), 1.33 ratio (in triploids), or 1.25 ratio (in tetraploids) relative to the rest of the chromosomes.

Bottleneck experiment. Ten replicate tetraploid populations were streaked onto YPD plates. Every 2 d (~22 generations) of growth, a single random colony was picked and streaked onto a new plate. Culture was frozen every 2 wk (~154 generations). This procedure of repeated bottlenecks ensured that each line had a low effective population size [29] ($N_e = 22$).

Rate of genome size evolution. A regression line was fit through the genome size data as a function of time. The y-intercept was constrained as the genome size at time zero and was thus the same for all ten (replicates × environment) lines of each ploidy. The mean slope was calculated for each of the six populations (ploidy × environment) as the mean of the slopes of the five replicate lines. A two-way ANOVA was performed to test for an interaction between ploidy and environments. *t*-tests were then performed to determine differences in the rate of genome size evolution between environments for each ploidy level. The assumption of normality was met in all cases ($p > 0.05$). All analyses were performed using JMP [30].

PCR. Yeast genomic DNA was isolated. PCR was run twice for all evolved (generation 1,766) haploid lines and the ancestral haploid lines using forward primers specific to the MATa (5′–CTCCACTT-CAAGTAAGAGTTTGGGT–3′) and MATalpha (5′–TTACTCA-CAGTTTGGCTCCGGTGT–3′) alleles and a common reverse primer (MAT 3′–R: 5′–GAACCGCATGGGCAGTTTACCTTT–3′). Amplification of DNA sequence was achieved by 30 cycles of DNA denaturation (96 °C for 1 min), primer annealing (55 °C, 1 min), and primer elongation (72 °C, 1 min) followed by a 5-min incubation at 72 °C after the final cycle. The haploid yeast strains YPH 499 (MATa) and YPH 500 (MATalpha) were used as controls to ensure the primers amplified the proper regions.

Supporting Information

Figure S1. Temporal Polymorphism for Genome Size across 1,800 Generations of Batch Culture

Found at DOI: 10.1371/journal.pgen.0020145.sg001 (725 KB PDF).

Figure S2. Genome Size Change across ~600 Generations of Batch Culture Evolution from a Replicate Experiment

Found at DOI: 10.1371/journal.pgen.0020145.sg002 (214 KB PDF).

Figure S3. Average Fluorescence Ratio by Chromosome from CGH between Lineages Early in the Experiment

Found at DOI: 10.1371/journal.pgen.0020145.sg003 (580 KB PDF).

Figure S4. Average Fluorescence Ratio by Chromosome from CGH between Lineages Late and Early in the Experiment

Found at DOI: 10.1371/journal.pgen.0020145.sg004 (631 KB PDF).

Figure S5. Average Fluorescence Ratio by Chromosome from CGH between Lineages Late in the Experiment

Found at DOI: 10.1371/journal.pgen.0020145.sg005 (624 KB PDF).

Figure S6. Sliding Window Analysis of the Arrays, using a Window Length of Ten Genes

Found at DOI: 10.1371/journal.pgen.0020145.sg006 (4.7 KB PDF).

Figure S7. Genome Size Measured from Ten Populations (B1–B10) Evolved by Repeated Bottlenecking for 566 Generations and Five Populations (S1–S5) Evolved through Batch Culture Transfers for 558 Generations

Found at DOI: 10.1371/journal.pgen.0020145.sg007 (542 KB PDF).

Protocol S1. Supplementary Material

Found at DOI: 10.1371/journal.pgen.0020145.sd001 (46 KB DOC).

Accession Numbers

The *Saccharomyces* Genome Database (SGD) (http://www.yeastgenome.org) accession numbers for Chromosomes 4, 5, and 12 are

NC__001136.8, NC__001137, and NC__001144.4, respectively; the accession numbers for *ENA1*, *ENA2*, and *ENA5* are YDR040C, YDR039C, and YDR038C, respectively.

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Author contributions. ACG, HJEC, AG, and SPO conceived and designed the experiments. ACG, HJEC, and AG performed the experiments. ACG, HJEC, and SPO analyzed the data. ACG and SPO wrote the paper.

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Competing interests. The authors have declared that no competing interests exist.

References

1. Cavalier-Smith T (1978) Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox. J Cell Sci 34: 247–278.
2. Gregory TR (2001) Coincidence, coevolution, or correlation? DNA content, cell size, and the C-value enigma. Biol Rev 76: 65–101.
3. Weiss RL, Kukora JR, Adams J (1975) The relationship between enzyme activity, cell geometry, and fitness in *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A 72: 794–798.
4. Petrov DA (2001) Evolution of genome size: New approaches to an old problem. Trends Genet 17: 23–28.
5. Lewis WH (1980) Polyploidy: Biological relevance. New York: Plenum Press. 583 p.
6. Otto SP, Whitton J (2000) Polyploid incidence and evolution. Annu Rev Genet 34: 401–437.
7. Paquin C, Adams J (1983) Frequency of fixation of adaptive mutations is higher in evolving diploid than haploid yeast populations. Nature 302: 495–500.
8. Perrot V, Richerd S, Valero M (1991) Transition from haploidy to diploidy. Nature 351: 315–317.
9. Orr HA, Otto SP (1993) Does diploidy increase the rate of adaptation? Genetics 136: 1475–1480.
10. Zeyl C, Vanderford T, Carter M (2003) An evolutionary advantage of haploidy in large yeast populations. Science 299: 555–558.
11. Anderson JB, Sirjusingh C, Ricker N (2004) Haploidy, diploidy and evolution of antifungal drug resistance in *Saccharomyces cerevisiae*. Genetics 168: 1915–1923.
12. Riehle MM, Bennett AF, Long AD (2001) Genetic architecture of thermal adaptation in *Escherichia coli*. Proc Natl Acad Sci U S A 98: 525–530.
13. Dunham MJ, Badrane H, Ferea T, Adams J, Brown PO, et al. (2002) Characteristic genome rearrangements in experimental evolution of *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A 99: 16144–16149.
14. Nasmyth K, Shore D (1987) Transcriptional regulation in the yeast life cycle. Science 237: 1162–1170.
15. Kassir Y, Simchen G (1991) Monitoring meiosis and sporulation in *Saccharomyces cerevisiae*. Methods in Enzym 194: 94–110.
16. Zeyl C, deVisser JA (2001) Estimates of the rate and distribution of fitness effects of spontaneous mutation in *Saccharomyces cerevisiae*. Genetics 157: 53–61.
17. Bennett RJ, Johnson AD (2003) Completion of a parasexual cycle in *Candida albicans* by induced chromosome loss in tetraploid strains. EMBO J 22: 2505–2515.
18. Mable BK, Otto SP (2001) Masking and purging mutations following EMS treatment in haploid, diploid and tetraploid yeast (*Saccharomyces cerevisiae*). Genet Res 77: 9–26.
19. Galitski T, Saldanha AJ, Styles CA, Lander ES, Fink GR (1999) Ploidy regulation of gene expression. Science 285: 251–254.
20. Mable BK (2001) Ploidy evolution in the yeast *Saccharomyces cerevisiae*: A test of the nutrient limitation hypothesis. J Evol Biol 14: 157–170.
21. Kuchler K, Sterne RE, Thorner J (1989) *Saccharomyces cerevisiae STE6* gene product: A novel pathway for protein export in eukaryotic cells. EMBO 8: 3973–3984.
22. Kassir Y, Hicks JB, Herskowitz I (1983) *SAD* Mutation of *Saccharomyces cerevisiae* is an extra **a** cassette. Mol Cell Biol 3: 871–880.
23. Selk E, Wills C (1998) Mismatch repair and the accumulation of deleterious mutations influence the competitive advantage of *MAT* (mating type) heterozygosity in the yeast *Saccharomyces cerevisiae*. Genet Res Camb 71: 1–10.
24. Nash R, Tokiwa G, Anand S, Erickson K, Futcher AB (1998) The WHI1+ gene of *Saccharomyces cerevisiae* tethers cell division to cell size and is a cyclin homolog. EMBO J 71: 4335–4346.
25. Watson JV, Chambers SH, Smith PJ. (1987) A pragmatic approach to the analysis of DNA histograms with a definable G1 peak. Cytometry 8: 1–8.
26. Pollack JR, Perou CM, Alizadeh AA, Elsen MB, Pergamenschikov A, et al. (1999) Genome-wide analysis of DNA copy-number changes using cDNA microarrays. Nat Genet 23: 41–46.
27. Hughes TR, Roberts CJ, Dai H, Jones AR, Meyer MR, et al. (2000) Widespread aneuploidy revealed by DNA microarray expression profiling. Nat Genet 25: 333–337.
28. Davis RW, Thomas M, Cameron J, St John TP, Scherer S, et al. (1980) Rapid DNA isolation for enzymatic and hybridization analysis. Methods Enzymol 65: 404–411.
29. Gerrish PJ, Wahl LM (2001) The probability that beneficial mutations are lost in populations with periodic bottlenecks. Evolution 55: 2606–2610.
30. SAS Institute (2005) JMP 5.1. Cary NC: SAS Institute Inc.

Protocol S1

Replicate Evolution Experiment

30 lines were initiated from the same ancestral stock (BM1N, BM2N, BM4N) and were evolved for 582 asexual generations using the same batch culture evolution methodology as the primary experiment. Results from FACScans are shown in Figure S1.

Analysis of Aneuploidy by CGH

Comparative genomic hybridization (CGH) was performed using 27 microarrays. Some arrays that appeared nosiy after intial analysis (e.g. array 10) were repeated. Otherwise, we attempted to maximize the number of lines hybridized onto the arrays using a round robin appraoch. All 25 successful arrays are shown (hybridization failed on arrays 17 and 25). Supplementary Figures S3 – S5 show the average fluorescence ratio of each chromosome obtained by CGH, along with 99.8% confidence intervals obtained by bootstrapping all of the gene ratio data from the particular chromosome.

Figure S3 compares genomes early in the experiment. Figure S3a compares two diploid lines from time point 0. The small amount of variation from the expected ratio of one is unlikely to be biologically significant. For example, consider the puzzling observation that numerically similar chromosomes (e.g., chromosomes 10 – 16) differ significantly from the expected ratio of one in the same direction (see green array 23 in Figure S3a). This observation is likely explained by the fact that genes on the same chromosome and from numerically adjacent chromosomes are more likely to be within the same row or column on the microarrays provided by the University Health Network. This interpretation is supported by the fact that the confidence intervals approximately double in length if we bootstrap by block (i.e., sample with replacement among the 48 blocks of 16×17 spots), a procedure that controls for the non-random design of the array within blocks. As aneuploidy should cause downward shifts to 0.75 (loss of one chromsomome in a tetraploid) or upward shifts to 1.25 (gain of one chromosome in a

tetraploid), we ignore small deviations from one (0.9 – 1.1) and discuss only larger departures.

Using this cut-off, chromosome 9 is at an average ratio of 1.14 in a tetraploid-diploid comparison at time 0 (Figure S3b). Even more variation is observed between the ps tetraploid line at cell generation ~100 and at generation 0 (Figure S3c), with chromosomes 2, 4, 5, and 9 at ratios 1.13, 1.29, 1.12, and 0.77, respectively. Similarly, substantial variation is observed between a ps tetraploid line at generation 200 and at generation 100, with chromosomes 3 and 10 at ratios 1.23 and 0.88, respectively. Most of these differences fall short of the expected ratios of 0.75 and 1.25 for tetraploid lines that gain or lose a chromosome. Two biologically interesting explanations are possible: partial aneuploidy or polymorphism within the lines compared by CGH. Sliding window analyses of these chromosomes showed no evidence of partial chromosome loss. Another possibility is that the lines had become polymorphic for chromosome content during the approximately 30 cell generations required to isolate significant DNA from cultures starting from a single cell.

Figure S4 illustrates CGH comparisons between lines isolated at generation 1766 and at generation 0. A majority of chromosomes exhibit fluorescence ratios near one, with some exceptions. Interestingly, aneuploidy always involved chromosome 9 (ratios of 0.86, 0.76, 0.71, 0.78, 0.64, and 0.70 from a - f), and often involved chromosome 4 (ratios of 1.32 and 1.28 from middle left to middle right).

Figure S5 illustrates CGH comparisons between different tetraploid lines isolated at generation 1766. Variation in chromosomal content is much less pronounced among comparisons made at the end of the evolution experiment than between lines at the start. In array 7, chromosomes 3, 9, 13 and 14 showed deviations beyond 0.9 – 1.1 (Figure S5a), as did chromosomes 2 and 4 in array 26 (Figure S5e). Yet these arrays also exhibited the pattern that numerically similar chromosomes differed in the same direction, suggesting that these deviations were artifacts. Indeed, sliding window analyses (Figure S6) did not show clear breaks at the chromosome boundaries, as expected from a chromosomal addition/deletion. The only consistent difference in

genomic content among the lines at the end of the experiment was chromosome 3 in the comparisons between line rs19 and R19 in arrays 13 and 15 (Figure S5c; average ratio = 1.15). Sliding window analyses of arrays 13, 15, and 18 suggest a partial deletion of chromosome 3 in line R19.

The interpretation of the microarrays is made complicated by the fact that the time 0 "controls" exhibited variation in chromosomal content (Figure S3). Nevertheless, several broad patterns are apparent in the results. Most importantly, the roughly triploid or diploid DNA content of the evolved lines detected by FACS analysis (Figure 1) is not consistent with random chromosome loss, as most of the lines analyzed from generation 1766 exhibited chromosomal ratios near one. However, the lines do exhibit aneuploid differences, appearing early in the experiment and often involving chromosome 9.

Analysis of Indels by CGH

CGH was also used to detect smaller deletions and insertions. We performed sliding window analyses using a window size of 10 genes to search for indels. We only focused on those windows that showed peaks or troughs that were outside of the range observed in 100 randomizations of the genome and that were consistent across multiple arrays involving the same lines. Using these criteria, three potential indels were identified. Multiple arrays allowed us to pinpoint the type of indel that occurred in case (i); our inferences for cases (ii) and (iii) are based on fewer arrays and are more tentative.

(i) Chromosome 4 insertion: A sliding window analysis of arrays 6 and 12 (designated *i* in Figure S6) demonstrated a higher ratio of the same segment on chromosome 4 (525434..538461) in qs-1766 relative to Q-1766. The genes involved, *KRS1*, *ENA1, ENA2,* and *ENA5*, were found at ratios in this region of 3.7 [2.5,5.6] and 3.1 [2.0-4.7] with 95% confidence intervals in brackets (based on a normal approximation using observed standard errors for the log-ratio data). Arrays 10, 11, and 14 also exhibit higher ratios in this region at 2.8 [1.2-6.4] (array 10), 4.0 [2.0-8.1] (array 11), and 2.6 [2.0-3.4] (array 14), indicating that qs-1766 carries an insertion. Consistent with this conclusion, the ratio in this region was not significantly different from one in array 9 (0.94 [0.73-1.13]) involving line Q-1766. We thus infer that an insertion has occurred in the qs high-

salt line, increasing the copy number of these genes several fold. Interestingly, *ENA5*, *ENA2*, and *ENA1* are P-type ATPases involved in Na+ and Li+ efflux (*Saccharomyces* Genome Database: http://www.yeastgenome.org/). Transposable element (TE) disruption within *ENA5* and *ENA1* has been shown to decrease salt tolerance[1]. We hypothesize that duplication of this region was selectively favoured in the high salt medium, improving the salt tolerance of the qs line.

(ii) Chromosome 5 indel: A sliding window analysis of array 13 and, to a lesser extent, array 15 (designated *ii* in Figure S6) indicated a lower ratio of a segment on chromosome 5 (spanning approximately sites 451560..487188 and encompassing ~18 genes) in R-1766 relative to rs-1766 (ratio 0.721 [0.68-0.74] in array 13; ratio 0.81 [0.78-0.83] in array 15). A similar trend was observed in array 18 (ratio of 0.68 [0.64-0.73] for R-1766 relative to a tetraploid line from time point 0), suggesting a deletion in R-1766, but the array was noisier (Figure S6q). Several genes within this region are known to reduce growth on YPD when deleted, either as heterozygous deletions *COG3* or as homozygous deletions (*COX15*, *OXA1*, *PET122*, *UBP3*, *BEM2*, *COG3*)[2], which argues against selective benefit to this deletion. Confirmation of the deletion and quantification of its fitness effects are warranted.

(iii) Chromosome 12 indel: A sliding window analysis of arrays 13 and 15 (designated *iii* in Figure S5) demonstrated a significantly lower ratio of a segment on chromosome 12 (469318..489930) in rs-1766 relative to R-1766. The genes involved, *ASP3-1*, *RDN5-3*, *ASP3-2*, *RDN5-4*, *RDN5-5, ASP3-4*, *RDN5-6*, were found at ratios of 0.60 [0.55-0.67] (array 13) and 0.64 [0.60-0.69] (array 15). No such pattern was observed in a sliding window analysis of R-1766 versus a time point 0 strain (1.3 [1.0-1.6], array 18), suggesting a deletion of this segment in rs-1766. These genes either have unknown function or are cell-wall proteins involved in asparagine catabolism (*Saccharomyces* Genome Database: http://www.yeastgenome.org/).

Interestingly, sliding window analyses suggest the presence of indels involving the same region of chromosome 12 in other genomic comparisons (asterisks in Figure S6), including line ps-1766 vs ancestral (arrays 3 and 4), ss-1766 vs S-1766 (array 16),

qs-1766 vs time 0 or Q-1766 (arrays 6, 12, 14). These signals were not, however, detectable across all arrays involving the same lines. This inconsistency might be due to the difficulties of detecting small indels using CGH and/or due to polymorphisms arising repeatedly in the preparation of DNA if this is an indel hotspot.

Bottleneck Experiment

Genome size was measured for ten initially tetraploid populations (B1-B10) evolved by repeated bottlenecking from colonies down to single cells for 566 cell generations. The genome size of five individuals were sampled from each line and compared to five individuals from each of the tetraploid populations evolved through batch culture transfers for 558 generations (from the orignial experiment). The population averages were significantly higher ($p=0.0003$; Figure S7) in the bottleneck line.

Calculating required fitness gains

We observed transitions from tetraploid (4n=64 chromosomes) to diploid (2n = 32 chromosomes) in replicate populations over the course of 100 – 200 generations (Figure 1e). We hypothesize that these transitions were driven by large-scale reductions in genome size, involving more than one chromosome at a time. Here, we show that it would take extremely strong selection if the transition instead involved a series of 32 mutations, each involving the loss of a single chromosome.

In the absence of sex and recombination, successive beneficial mutation can only spread to fixation if they arise sequentially in the same background. Following Crow & Kimura[3], we calculate the expected number of generations between the appearance of the first beneficial mutation in an asexual population and the appearance of an individual that carries two beneficial mutations, counting only those beneficial mutations that survive stochastic loss while rare. At generation t, we expect $N\,p(t)\,\mu$ second mutations to occur in an individual carrying the first mutation, where $p(t)$ is the frequency of the

first mutation, N is the population size, and μ is the rate at which the second mutation occurs. Of these second mutations, only a fraction will survive loss while rare[4]; when selection is strong, the fraction surviving is approximately $1-e^{-2s}$.

We can then ask how many generations, g, must pass before the cumulative expected number of second mutations (specifically, beneficial mutations that arise in an individual carrying the first mutation and surviving loss while rare) rises above one. That is, we find g such that $\int_0^g N\,p(t)\,\mu\left(1-e^{-2s}\right)\mathrm{d}t > 1$. Measuring time from the appearance of the first mutation, the frequency of the first mutation obeys the classical model of selection: $p(t) = (1+s)^t\,p(0) \Big/ \left(1-p(0)+(1+s)^t\,p(0)\right)$, where $p(0) = 1/N$. Solving the integral, we find:

$$g = \frac{\ln\left(N\left(1+s\right)^{1/\left(N\mu\left(1-e^{-2s}\right)\right)} - N + 1\right)}{\ln(1+s)} \qquad \text{(S1)}$$

Equation (S1) gives the expected number of generations until two beneficial mutations are present together in the same individual. Consequently, $31 \times g$ generations must pass, on average, until an individual would arise that had lost 32 chromosomes by mutation, if each mutation were to occur independently. In our experiment, the effective population size was on the order of $N = 10^7$. Using this parameter value and a relatively high mutation rate of $\mu = 10^{-5}$, we find that the selection coefficient, s, must be greater than 5.5 to account for a transition from tetraploidy to diploidy in 200 generations by the loss of one chromosome at a time. This corresponds to a 550% increase in fitness every time a chromosome is lost. This estimated selection coefficient is not sensitive to the exact value of the population size, and much higher mutation rates are needed to cause the requisite selection coefficient to drop substantially (e.g., $\mu > 10^{-3}$ for $s < 2$). Although we have treated the population size as constant, accounting for fluctuations in population size only serves to increase the selection coefficient needed to account for 32 independent mutations arising and spreading in succession (details available upon request). It is

highly implausible that the loss of each chromosome is accompanied by such extreme selection, leading us to conclude that the transition from tetraploidy to diploidy did not occur by a series of independent mutations, losing one chromosome at a time. Instead, our data support the hypothesis that multiple chromosomes were lost simultaneously. Whether or not this was precipitated by a mutational event (e.g., losing one chromosome increases the mutation rate to loss of non-homologous chromosomes) remains unknown.

Supplementary Information References:

1. de Jesus Ferreira, M.C., Bao, X., Laizé, V., & Hohmann, S. Transposon mutagenesis reveals novel loci affecting tolerance to salt stress and growth at low temperature. *Curr Genet.* **40**:27-39 (2001)

2. Deutschbauer A. M *et al.* Mechanisms of haploinsufficiency revealed by genome-wide profiling in yeast. *Genetics* **169**:1915-25 (2005)

3. Crow, J.F. & Kimura, M. Evolution in sexual and asexual populations. *Am Nat* **99**: 439-450 (1965)

4. Haldane, J.B.S. The mathematical theory of natural and artificial selection. *Proc. Camb. Philos. Soc.* **23**: 838-844 (1927)

Supplementary Figure S1: Temporal polymorphism for genome size across 1800 generations of batch culture. One line from each hapoid and tetraploid population was picked for more in depth analysis. 10 colonies at each time point were assayed for genome size (closed circles). The line and open circles denote the original, single colony data presented in Figure 1.

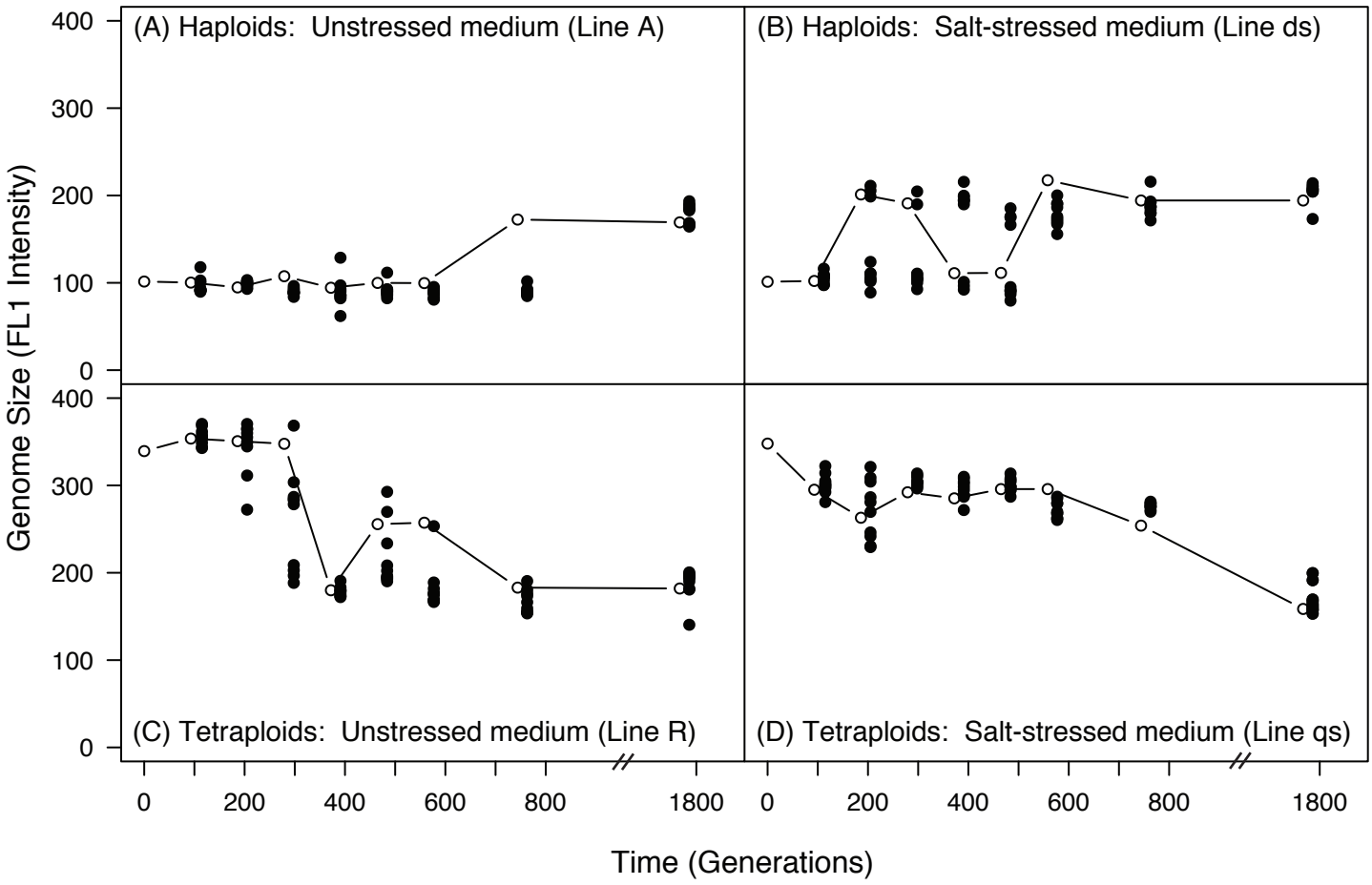
Supplmentary Figure S2: Genome size change across ~600 generations of batch culture evolution from a replicate experiment. FL1 is a linear scale of dye fluorescence as measured by flow cytometry (FACS). The five lines on each graph represent the five replicate lines evolved independently. Note the similar pattern of genome size change depicted in this figure and the first ~600 generations of Figure 1.

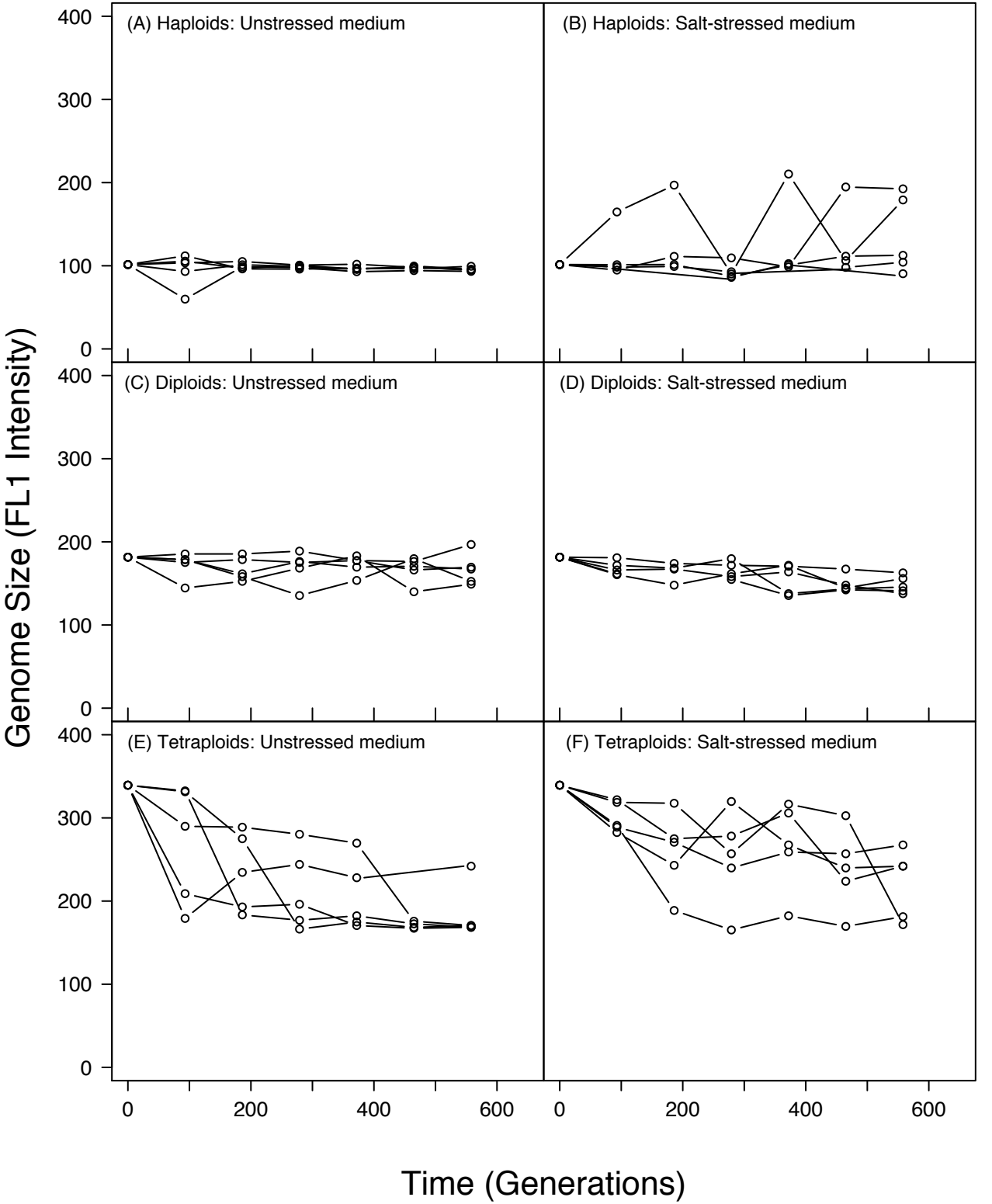
Supplementary Figure S3: Average fluorescence ratio by chromosome from a comparative genomic hybridization between lineages early in the experiment. Repeated arrays are illustrated in different colors. Chromosomes with average fluorescence ratios outside of 0.9 – 1.1 are indicated in blue text.

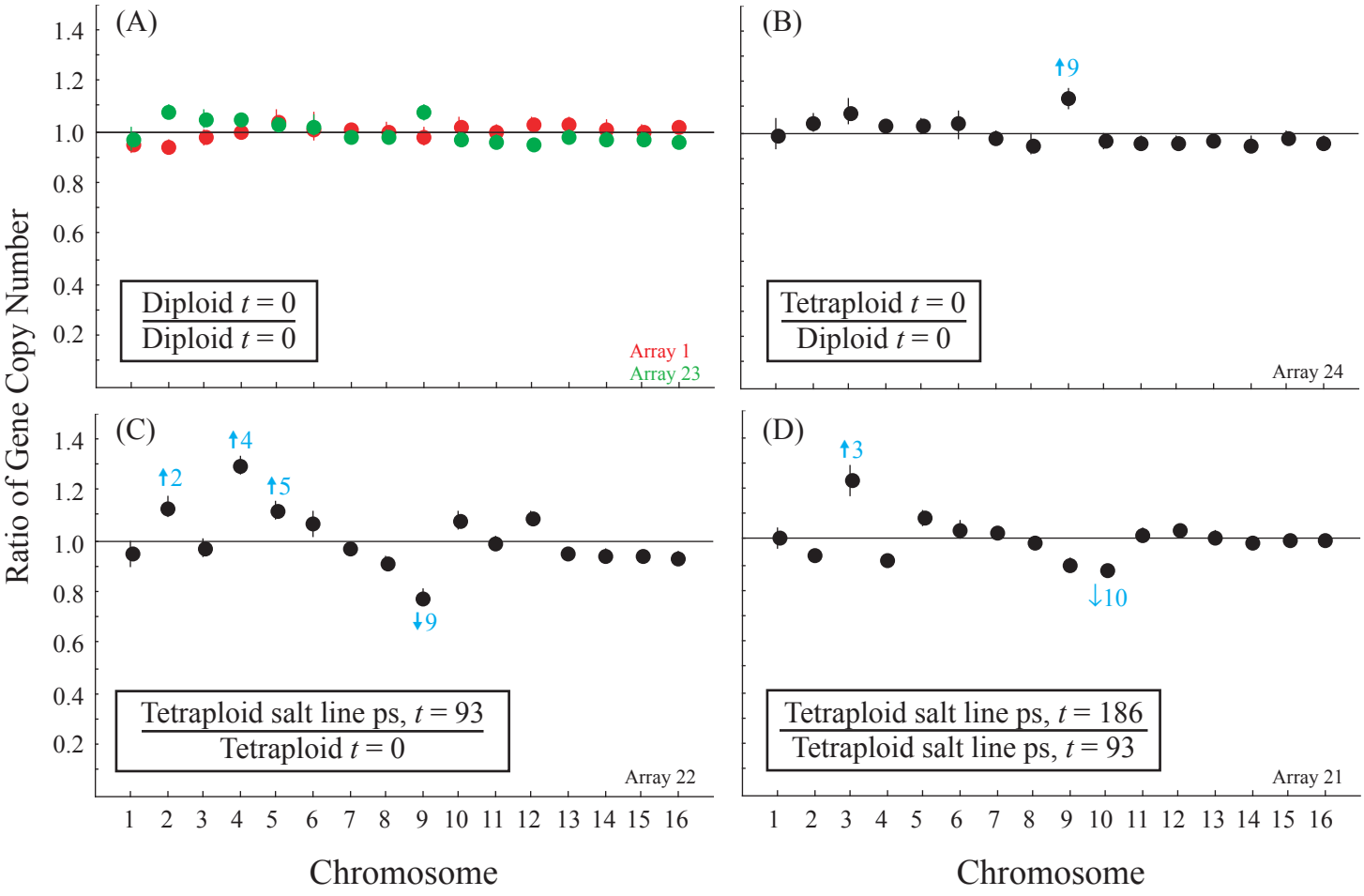
Supplementary Figure S4: Average fluorescence ratio by chromosome from a comparative genomic hybridization between lineages late and early in the experiment. Repeated arrays are illustrated in different colors. Chromosomes with average fluorescence ratios outside of 0.9 – 1.1 are indicated in blue text.

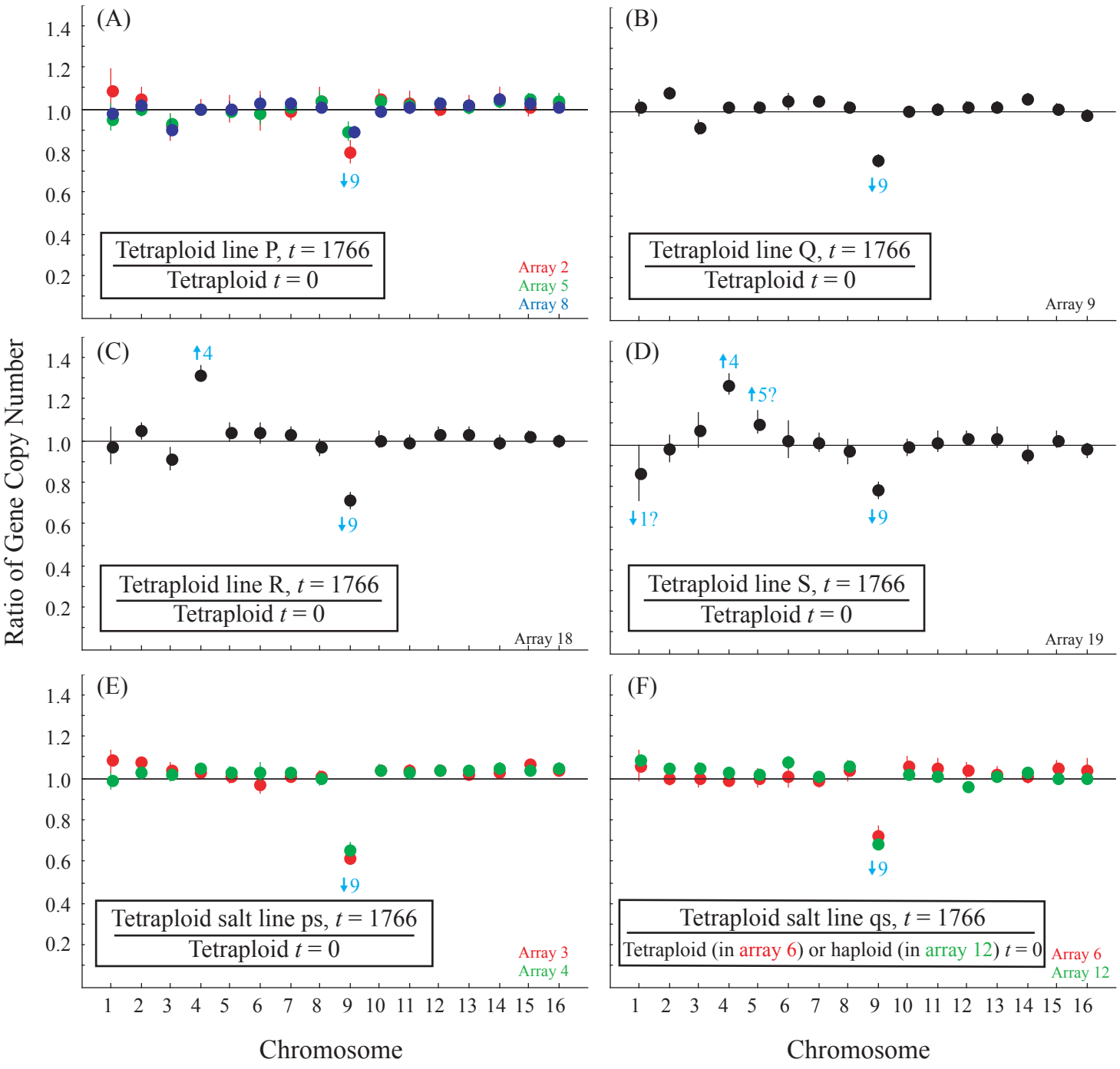
Supplementary Figure S6: Fluorescence ratios for sliding window analyses of the arrays, using a window length of 10 genes. Dashed lines represent the maximum and minimum observed in sliding windows of 100 randomized genomes. Blue lines represent chromosomal differences inferred from Figures S1 – S3. Letters i - iii (and *) represent indels discussed in the text.

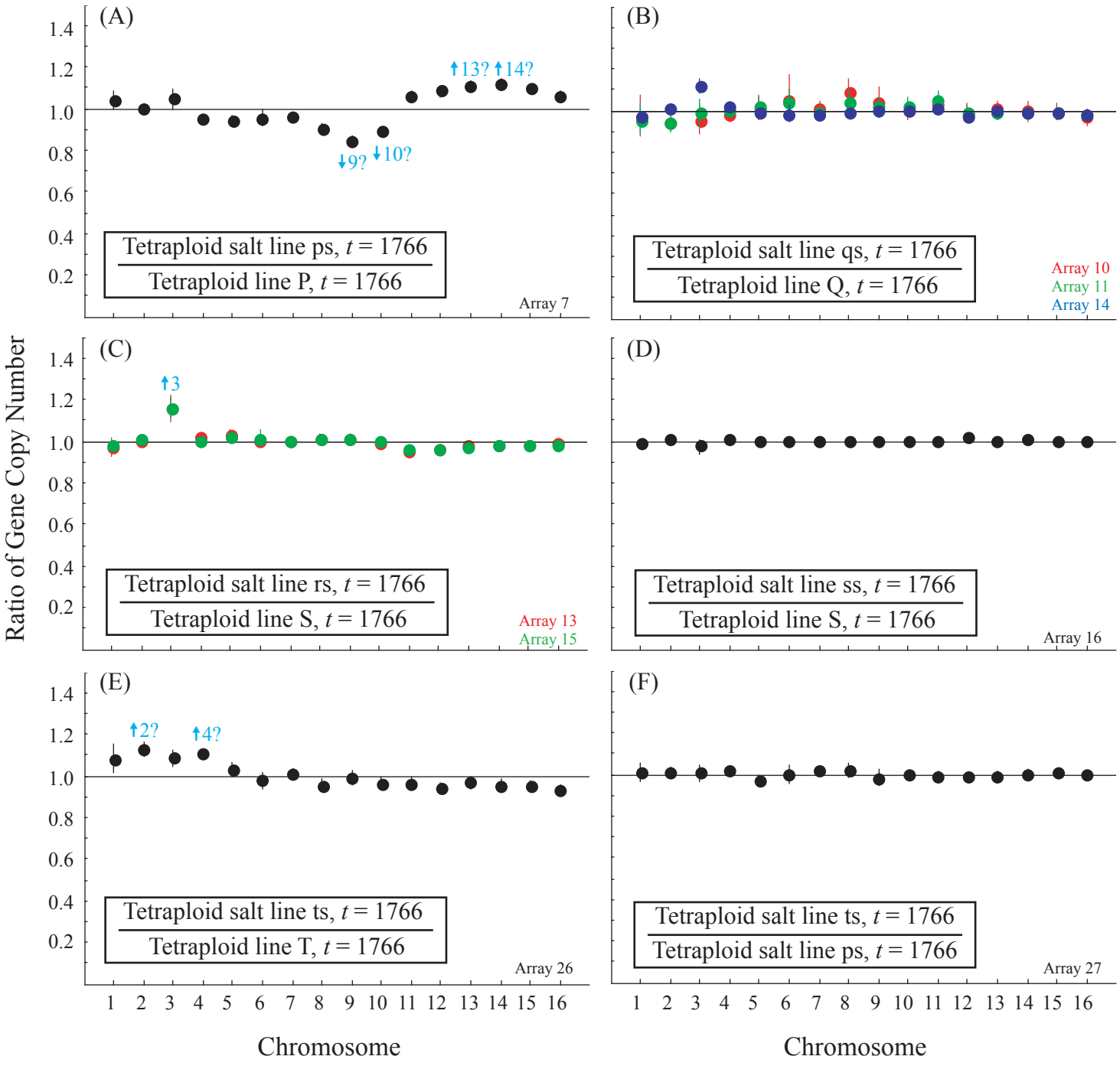
Supplementary Figure S7: Genome size measured from ten populations (B1-B10) evolved by repeated bottlenecking for 566 generations and five populations (S1-S5) evolved through batch culture transfers for 558 generations. Five individuals were sampled from each population (circles). The population averages were significantly higher ($p=0.0003$) for the bottleneck lines (B1-B10) than the original batch culture lines (S1-S5). The two arrows pointing at the y-axis indicate the average genome size (±2.5) of five individuals sampled from the ancestral diploid and tetraploid stocks.

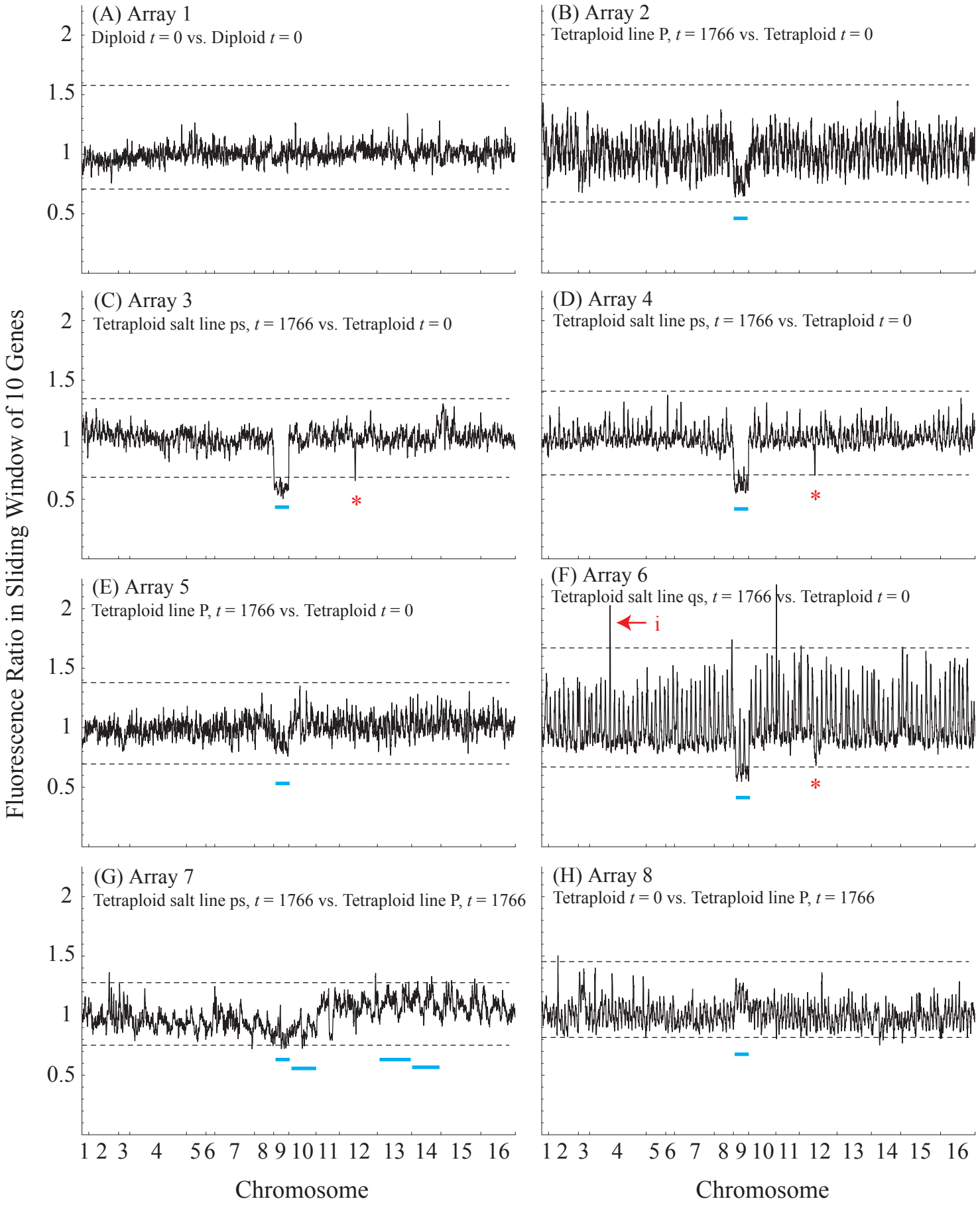

(A) Haploids: Unstressed medium (Line A)
(B) Haploids: Salt-stressed medium (Line ds)
(C) Tetraploids: Unstressed medium (Line R)
(D) Tetraploids: Salt-stressed medium (Line qs)
Genome Size (FL1 Intensity)
Time (Generations)
0
100
200
300
400
0
200
400
600
800
1800

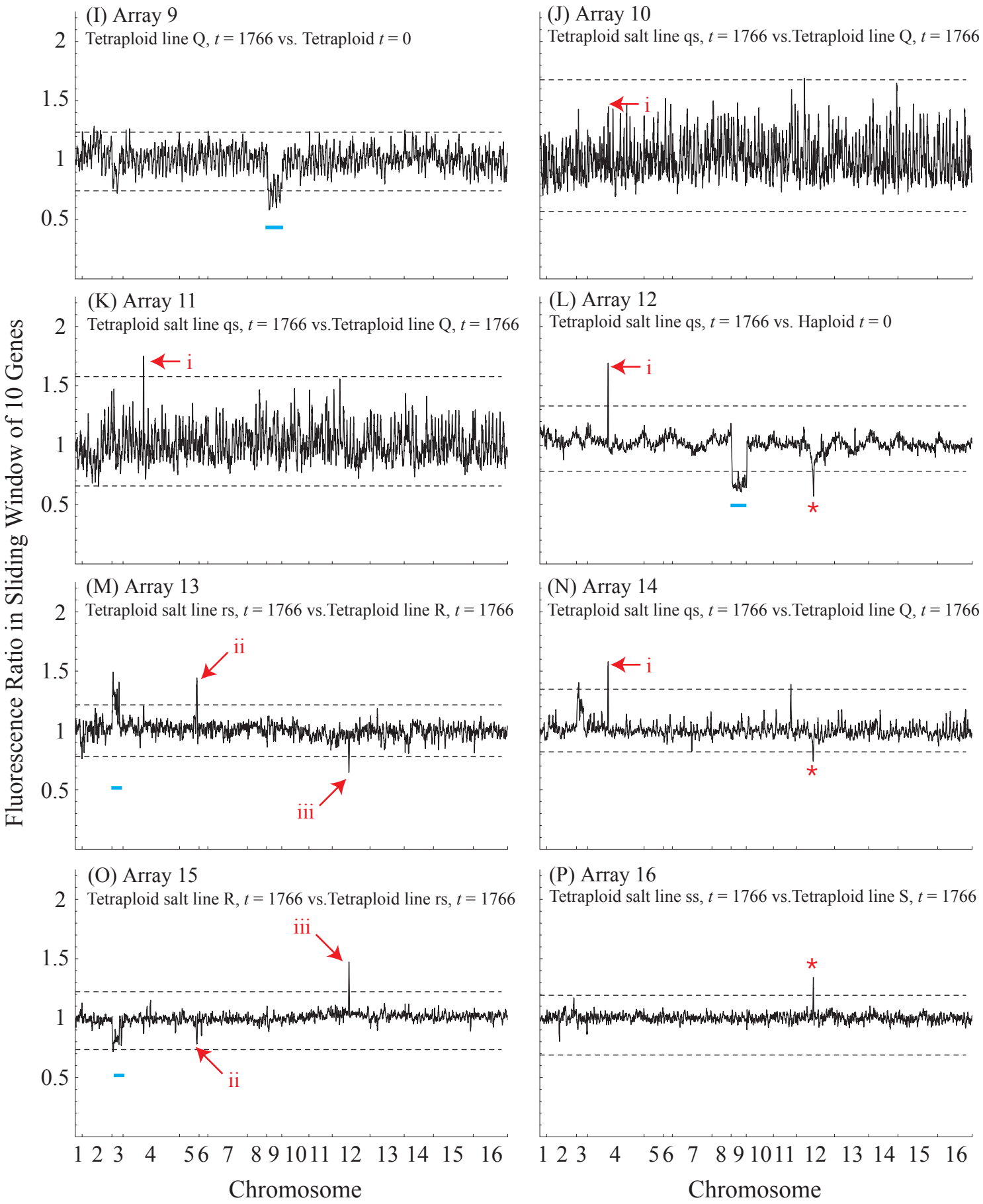

(A) Haploids: Unstressed medium
(B) Haploids: Salt-stressed medium
(C) Diploids: Unstressed medium
(D) Diploids: Salt-stressed medium
(E) Tetraploids: Unstressed medium
(F) Tetraploids: Salt-stressed medium
Genome Size (FL1 Intensity)
Time (Generations)
0
100
200
300
400
600

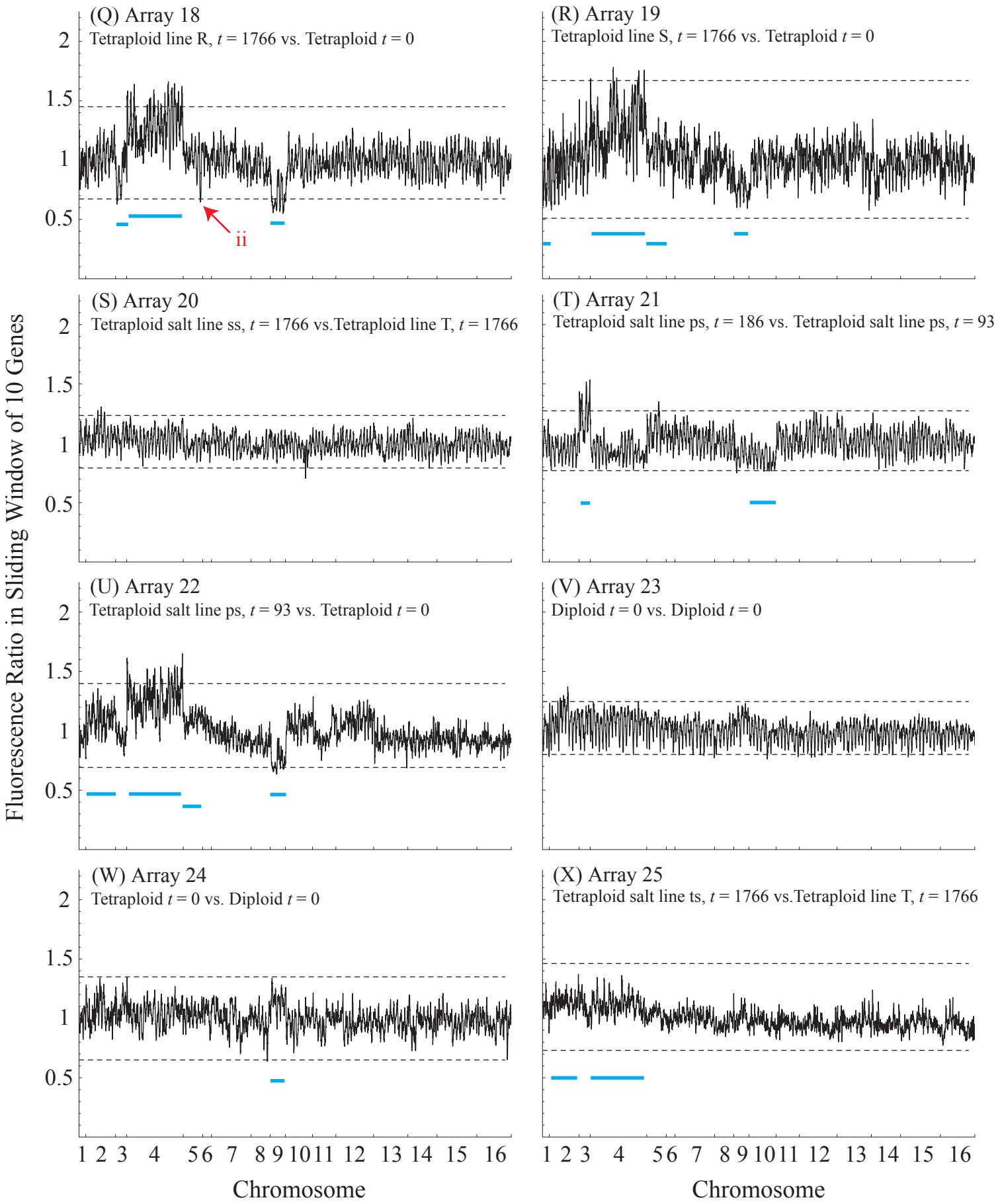

(A)
Diploid t = 0
Diploid t = 0
Array 1
Array 23
(B)
↑9
Tetraploid t = 0
Diploid t = 0
Array 24
(C)
↑2
↑4
↑5
↓9
Tetraploid salt line ps, t = 93
Tetraploid t = 0
Array 22
(D)
↑3
↓10
Tetraploid salt line ps, t = 186
Tetraploid salt line ps, t = 93
Array 21
Ratio of Gene Copy Number
1.4
1.2
1.0
0.8
0.6
0.4
0.2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Chromosome

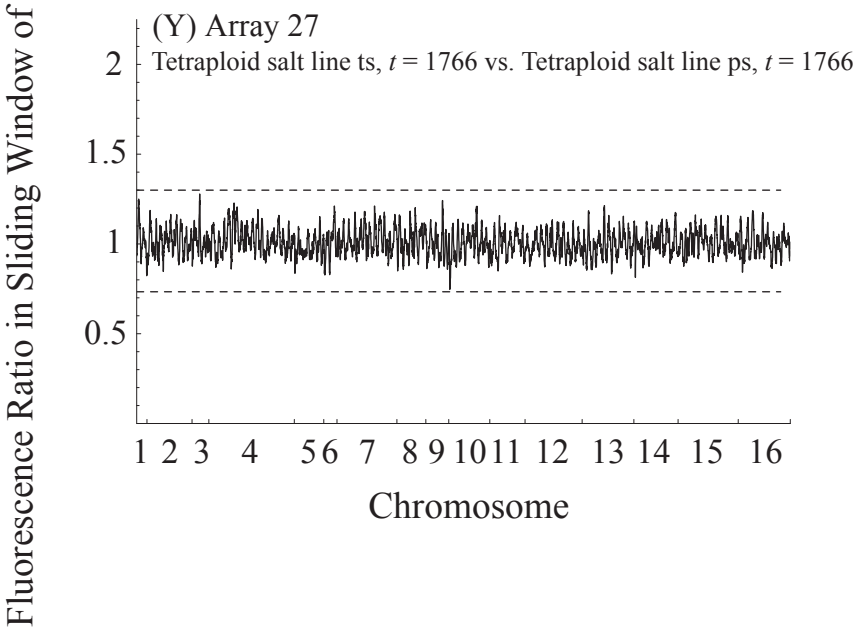

(A)
Tetraploid line P, t = 1766
Tetraploid t = 0
Array 2
Array 5
Array 8
↓9
(B)
Tetraploid line Q, t = 1766
Tetraploid t = 0
Array 9
↓9
(C)
Tetraploid line R, t = 1766
Tetraploid t = 0
Array 18
↑4
↓9
(D)
Tetraploid line S, t = 1766
Tetraploid t = 0
Array 19
↑4
↑5?
↓1?
↓9
(E)
Tetraploid salt line ps, t = 1766
Tetraploid t = 0
Array 3
Array 4
↓9
(F)
Tetraploid salt line qs, t = 1766
Tetraploid (in array 6) or haploid (in array 12) t = 0
Array 6
Array 12
↓9
Ratio of Gene Copy Number
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Chromosome
Chromosome

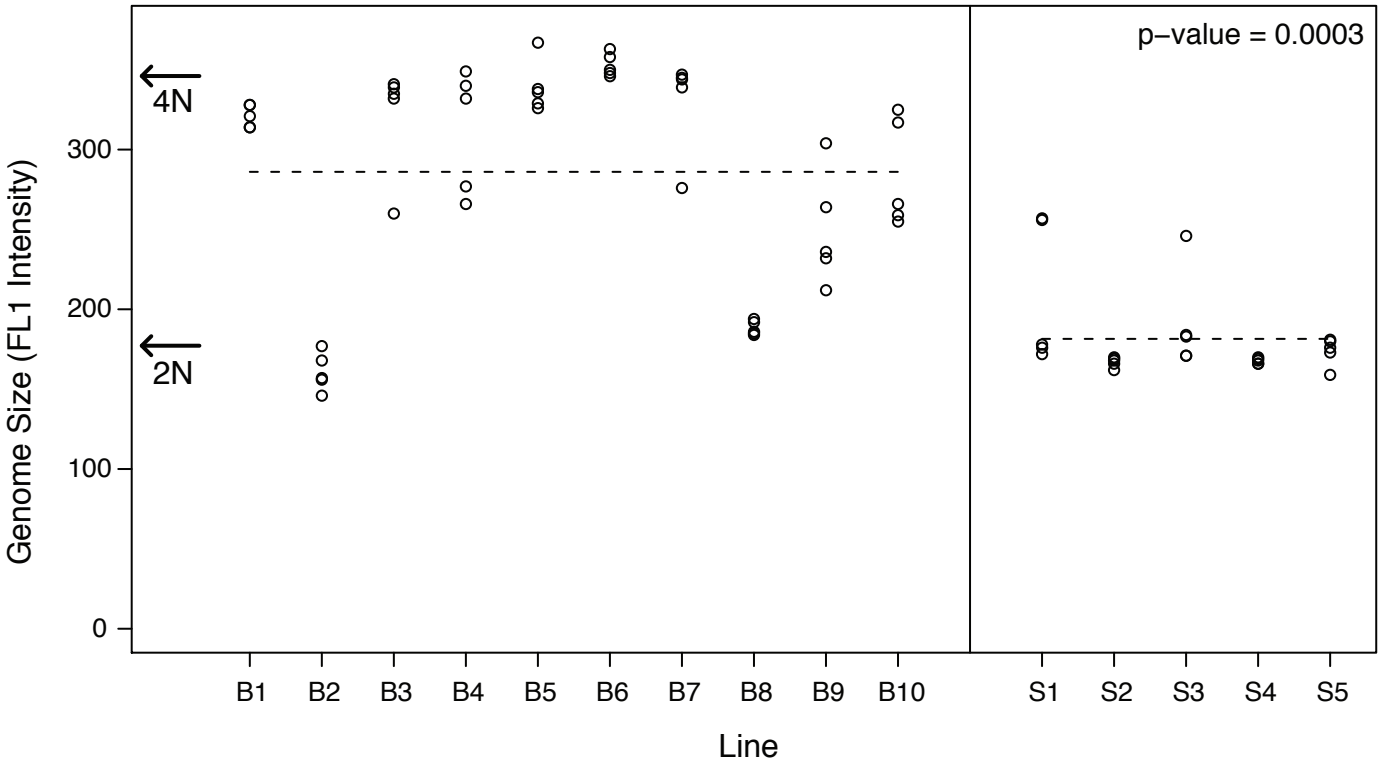

(A)
↑13? ↑14?
↓9? ↓10?
Tetraploid salt line ps, t = 1766
Tetraploid line P, t = 1766
Array 7
(B)
Tetraploid salt line qs, t = 1766
Tetraploid line Q, t = 1766
Array 10
Array 11
Array 14
(C)
↑3
Tetraploid salt line rs, t = 1766
Tetraploid line S, t = 1766
Array 13
Array 15
(D)
Tetraploid salt line ss, t = 1766
Tetraploid line S, t = 1766
Array 16
(E)
↑2?
↑4?
Tetraploid salt line ts, t = 1766
Tetraploid line T, t = 1766
Array 26
(F)
Tetraploid salt line ts, t = 1766
Tetraploid salt line ps, t = 1766
Array 27
Ratio of Gene Copy Number
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Chromosome
Chromosome


(A) Array 1
Diploid t = 0 vs. Diploid t = 0
(B) Array 2
Tetraploid line P, t = 1766 vs. Tetraploid t = 0
(C) Array 3
Tetraploid salt line ps, t = 1766 vs. Tetraploid t = 0
(D) Array 4
Tetraploid salt line ps, t = 1766 vs. Tetraploid t = 0
(E) Array 5
Tetraploid line P, t = 1766 vs. Tetraploid t = 0
(F) Array 6
Tetraploid salt line qs, t = 1766 vs. Tetraploid t = 0
i
(G) Array 7
Tetraploid salt line ps, t = 1766 vs. Tetraploid line P, t = 1766
(H) Array 8
Tetraploid t = 0 vs. Tetraploid line P, t = 1766
Fluorescence Ratio in Sliding Window of 10 Genes
2
1.5
1
0.5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Chromosome


(I) Array 9
Tetraploid line Q, t = 1766 vs. Tetraploid t = 0
(J) Array 10
Tetraploid salt line qs, t = 1766 vs.Tetraploid line Q, t = 1766
(K) Array 11
Tetraploid salt line qs, t = 1766 vs.Tetraploid line Q, t = 1766
(L) Array 12
Tetraploid salt line qs, t = 1766 vs. Haploid t = 0
(M) Array 13
Tetraploid salt line rs, t = 1766 vs.Tetraploid line R, t = 1766
(N) Array 14
Tetraploid salt line qs, t = 1766 vs.Tetraploid line Q, t = 1766
(O) Array 15
Tetraploid salt line R, t = 1766 vs.Tetraploid line rs, t = 1766
(P) Array 16
Tetraploid salt line ss, t = 1766 vs.Tetraploid line S, t = 1766
Fluorescence Ratio in Sliding Window of 10 Genes
2
1.5
1
0.5
i
ii
iii
*
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Chromosome


(Q) Array 18
Tetraploid line R, t = 1766 vs. Tetraploid t = 0
ii
(R) Array 19
Tetraploid line S, t = 1766 vs. Tetraploid t = 0
(S) Array 20
Tetraploid salt line ss, t = 1766 vs.Tetraploid line T, t = 1766
(T) Array 21
Tetraploid salt line ps, t = 186 vs. Tetraploid salt line ps, t = 93
(U) Array 22
Tetraploid salt line ps, t = 93 vs. Tetraploid t = 0
(V) Array 23
Diploid t = 0 vs. Diploid t = 0
(W) Array 24
Tetraploid t = 0 vs. Diploid t = 0
(X) Array 25
Tetraploid salt line ts, t = 1766 vs.Tetraploid line T, t = 1766
Fluorescence Ratio in Sliding Window of 10 Genes
2
1.5
1
0.5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Chromosome


(Y) Array 27
Tetraploid salt line ts, t = 1766 vs. Tetraploid salt line ps, t = 1766
Fluorescence Ratio in Sliding Window of 10 Genes
2
1.5
1
0.5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Chromosome


p−value = 0.0003
4N
2N
Genome Size (FL1 Intensity)
0
100
200
300
B1
B2
B3
B4
B5
B6
B7
B8
B9
B10
S1
S2
S3
S4
S5
Line