

# Aneuploidy can be an evolutionary detour on the path to adaptation

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## Abstract

16 Aneuploidy is common in eukaryotes, often leading to decreased fitness. However, evidence  
from fungi and human tumour cells suggests that specific aneuploidies can be beneficial under  
18 stressful conditions and facilitate adaptation. In a previous evolutionary experiment with yeast,  
populations evolving under heat stress became aneuploid, only to later revert to euploidy after  
20 beneficial mutations accumulated. It was therefore suggested that aneuploidy is a "stepping stone"  
on the path to adaptation. Here, we test this hypothesis. First, we apply DNA sequencing  
22 to show that mutant alleles common in aneuploid cells are uncommon in the evolved euploid  
population. Second, we use Bayesian inference to fit an evolutionary model with both aneuploidy  
24 and mutation the experimental results. We then predict the genotype frequency dynamics during  
the experiment, demonstrating that most of the evolved euploid population likely did not descend  
26 from aneuploid cells, but rather from the euploid wildtype population. Our model shows how the  
beneficial mutation supply—the product of population size and beneficial mutation rate—determines  
28 the evolutionary dynamics: with low supply, much of the evolved population descends from  
aneuploid cells; but with high supply, beneficial mutations are generated fast enough to outcompete  
30 aneuploidy due to its inherent fitness cost. Together, our results suggest that despite its potential  
fitness benefits under stress, aneuploidy can be an evolutionary "detour" rather than a "stepping  
32 stone": it can delay, rather than facilitate, the adaptation of the population, and cells that become  
aneuploid may leave less descendants compared to cells that remain diploid.

## 34 Introduction

Aneuploidy is an imbalance in the number of chromosomes in the cell: an incorrect karyotype.  
36 Evidence suggests aneuploidy is very common in eukaryotes, e.g. animals<sup>49,38,2</sup>, and fungi<sup>41,73,45,62</sup>.  
Aneuploidy has been implicated in cancer formation, progression, and drug resistance<sup>4,51,49,48,23,34</sup>.  
38 It is also common in protozoan pathogens of the *Leishmania* genus, a major global health concern<sup>36</sup>,  
and contributes to the emergence of drug resistance<sup>52</sup> and virulence<sup>37</sup> in fungal pathogens, which  
40 are under-studied<sup>47</sup>, despite infecting a billion people per year, causing significant morbidity in >150  
million and death in >1.5 million people per year<sup>52,47</sup>.  
42 Experiments with human and mouse embryos found that most germ-line aneuploidies are lethal. Ane-  
uploidies are also associated with developmental defects and lethality in other multicellular organ-  
44 isms<sup>55</sup>. For example, aneuploid mouse embryonic cells grow slower than euploid cells<sup>67</sup>. Similarly,  
in unicellular eukaryotes growing in benign conditions, aneuploidy usually leads to slower growth and  
46 decreased overall fitness, in part due to proteotoxic stress due to increased expression, gene dosage  
imbalance, and hypo-osmotic-like stress<sup>39,65,41,55,50,27,72,66,68,46</sup>.  
48 However, aneuploidy can be beneficial under stressful conditions due to the wide range of phenotypes  
it can produce, some of which are advantageous<sup>41,68</sup>. Indeed, in a survey of 1,011 yeast strains,  
50 aneuploidy has been detected in about 19%<sup>42</sup>. Thus, aneuploidy can lead to rapid adaptation in  
unicellular eukaryotes<sup>17,64,21,44</sup>, as well as to rapid growth of somatic tumour cells<sup>51,57</sup>. For example,  
52 aneuploidy in *Saccharomyces cerevisiae* facilitates adaptation to a variety of stressful conditions like  
heat and pH<sup>70</sup>, copper<sup>8,17</sup>, salt<sup>11,46</sup>, and nutrient limitation<sup>12,19,1</sup>, with similar results in *Candida*  
54 *albicans*<sup>68</sup>. Importantly, aneuploidy can also lead to drug resistance in pathogenic fungi such as  
*C. albicans*<sup>54,53,16</sup> and *Cryptococcus neoformans*<sup>58</sup>, which cause candidiasis and meningoencephalitis,  
56 respectively. Although we focus here on aneuploidy, a similar phenomena of adaptation via gene  
duplication or amplification has been observed in yeast<sup>33</sup>, bacteria<sup>60</sup>, and DNA viruses<sup>13</sup>.  
58 Yona et al.<sup>70</sup> demonstrated experimentally the importance of aneuploidy in adaptive evolution. They  
evolved populations of *S. cerevisiae* under strong heat stress. The populations adapted to the heat stress  
60 within 450 generations, and this adaptation was determined to be due a duplication of Chromosome  
III. Later on, after more than 1,500 generations, the populations reverted back to an euploid state, while  
62 remaining adapted to the heat stress. Aneuploidy was therefore suggested to be a *transient adaptive*  
*solution*, because it can rapidly appear and fixate in the population under stressful conditions, and can  
64 then be rapidly lost when the cost of aneuploidy outweighs its benefit—after the stress is removed,  
or after "refined" beneficial mutations appear and fixate<sup>70</sup>. Furthermore, it has been suggested that

66 aneuploidy is an evolutionary "stepping stone" that facilitates future adaptation by genetic mutations,  
which require more time to evolve<sup>70,69</sup>.

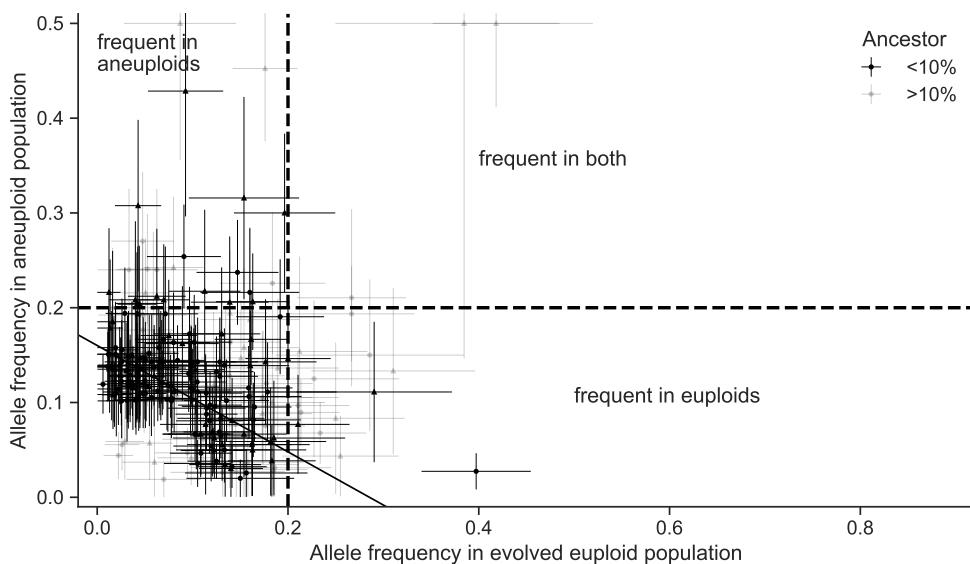
68 Here, we test the hypothesis that aneuploidy is a an *evolutionary stepping stone* that facilitates adaptive  
evolution by genetic mutations Yona et al.<sup>69</sup>. First, we sequenced the genomes of evolved populations  
70 reported in<sup>70</sup> and analyzed their mutant allele frequencies to assess if the evolved euploid cells are  
descended from aneuploid cells. Second, we develop an evolutionary genetic model and fit it to  
72 the experimental results of Yona et al.<sup>70</sup> in order to predict the genotype frequency dynamics in the  
experimental populations, thereby estimating the frequency of evolved euploid cells that descended  
74 from aneuploid cells. Our results show that aneuploidy reached high frequencies in the experimental  
populations, but nevertheless, the majority of cells in the evolved euploid population likely did not  
76 descend from aneuploid cells, but rather directly from wild-type euploid cells. These results suggest  
that at the lineage level, aneuploidy may be an evolutionary detour, rather than a stepping stone, on  
78 the path to adaptation.

## Results

80 In the heat-stress experiment of Yona et al.<sup>70</sup>, four populations of *S. cerevisiae* evolved under 39 °C.  
Aneuploidy fixed in all four experimental repetitions in the first 450 generations. Two of the repetitions,  
82 marked *H2* and *H4*, carried no large-scale duplications other than a Chromosome III trisomy. These  
two repetitions continued to evolve under the same conditions, wherein aneuploidy was eliminated by  
84 generation 1,700 and 2,350 in *H4* and *H2*, respectively.

**Empirical frequencies of mutant alleles.** For each of two evolved populations (*H2* and *H4*) we  
86 sequenced the ancestral diploid population (generation 0), the aneuploid population (generation 450),  
and the evolved euploid population (generation 1,700 or 2,350) to estimate the mutant allele frequencies  
88 (Tables S1 and S2). Overall, between 100 and 173 mutant alleles were detected with at least a single  
read in the six populations that were sampled. Disregarding 45 and 40 alleles that were present in  
90 the ancestral populations at a frequency >10%, the aneuploid and euploid populations carried a large  
number of mutant alleles: 82 and 95, respectively, in repetition *H2*, and 60 and 66 in repetition *H4*.  
92 37 mutant alleles were shared between the two evolved euploid populations, and their frequencies  
were correlated (Spearman's correlation:  $r = 0.4$ ,  $p = 0.15$ ). Of these 37, only two were identified  
94 in the Saccharomyces Genome Database<sup>7</sup>: YDR034C-A on Chromosome IV and YGR265W on  
Chromosome VII. Neither has a known function, and the former was previously identified as slightly  
96 decreasing heat tolerance<sup>25</sup>.

Surprisingly, out of all these mutant alleles, none was present at a frequency >20% in both the  
 98 aneuploid and the evolved euploid populations. More importantly, a high mutant allele frequency  
 in the aneuploid population was associated with a low frequency in the evolved euploid population,  
 100 and vice-versa (Spearman's correlation coefficient  $\rho = -0.64$  and  $-0.66$  in the two experimental  
 repetitions; Figure 1), suggesting that mutant alleles frequent in the aneuploid populations decreased  
 102 in frequency when aneuploidy was lost. These results suggest evolved euploid cells are unlikely to  
 have descended from aneuploid cells.



**Figure 1: Frequencies of mutant alleles in the experimental populations are negatively correlated.** Frequencies of mutant alleles when trisomy was widespread in the population (y-axis) and after it was eliminated (x-axis) in two experimental repetitions (circles for *H2* and triangles for *H4*) from Yona et al.<sup>70</sup>. Mutant alleles with >20% in the aneuploid population were <20% in the euploid population, and vice versa (the upper-right quadrant is empty), suggesting that the majority of evolved euploid cells did not descend from the most common aneuploid genotypes. Alleles with frequency below and above 10% in the ancestral populations are in black and gray, respectively. Solid black line is a linear orthogonal distance regression line (slope= $-0.559$ , intercept=0.164; a regression through alleles that reach at least 20% in one of the populations has slope= $-0.645$  and intercept=0.297). Dashed vertical and horizontal lines show allele frequencies of 20%. Error bars show SEM (standard error of the mean) assuming the number of reads is Binomially distributed; the SEM may be large when the total number of reads is small. For the 18 mutant alleles with high frequency in the aneuploid populations (>20%), the highest frequencies in the euploid populations were 15.4%, 16%, 16.3% and 19.6% (the rest were below 15%). Similarly, for the 48 mutant alleles with high frequency in the evolved euploid populations, the highest frequencies in the aneuploid populations were 2.7%, 7.7%, and 11.1% (the rest were below 1%).

104 **Evolutionary genetic model.** To explore the dynamics during the evolutionary experiments, we  
developed an evolutionary genetic model, fitted the model to empirical data, and used it to predict the  
106 genotype frequency dynamics, or specifically, the fraction of the evolved euploid population descended  
from aneuploid cells.

108 The model includes the effects of natural selection, genetic drift, aneuploidy, and mutation, and follows  
a population of cells characterized by their genotype: euploid wild-type,  $2n$ , is the ancestral diploid  
110 genotype; euploid mutant,  $2n^*$ , has a diploid karyotype and a single beneficial mutation; aneuploid  
wild-type,  $2n+1$ , has an extra chromosome due to a chromosome duplication event; and aneuploid  
112 mutant,  $2n+1^*$ , has an extra chromosome (like  $2n+1$ ) and a beneficial mutation (like  $2n^*$ ). Fitness  
values of the different genotypes are denoted by  $w_{2n}$ ,  $w_{2n^*}$ ,  $w_{2n+1}$ , and  $w_{2n+1^*}$ , and the rate of mutation  
114 and aneuploidy are denoted by  $\mu$  and  $\delta$ , respectively. See Figure 2 for an illustration of the model.

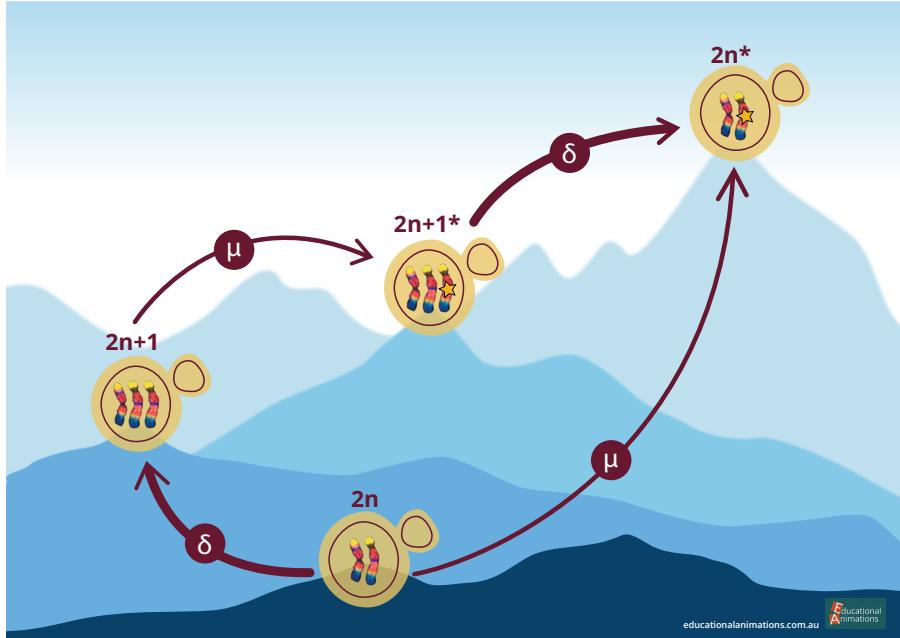
We fitted this model to the experimental results<sup>70</sup> – time for fixation (>95%) and for loss (<5%) of  
116 aneuploidy – using approximate Bayesian computation with sequential Monte Carlo (ABC-SMC)<sup>59</sup>,  
thereby inferring the model parameters: rates aneuploidy and mutation and the fitness of all genotypes.

118 We then sampled posterior predictions for the genotype frequency dynamics using the estimated  
parameter values and compared different versions of the model to test additional hypotheses about the  
120 evolutionary process.

**Estimated rates and fitness effects of aneuploidy and mutation.** We inferred the posterior distribution  
122 of model parameters (Figure 3). We report parameter estimates using the MAP (maximum a  
posteriori) and providing the 50% HDI (highest density interval) in square brackets. See Supplemen-  
124 tary Material for sensitivity analysis.

The estimated beneficial mutation rate is  $\mu = 2.965 \cdot 10^{-6}$  [ $2.718 \cdot 10^{-7} - 3.589 \cdot 10^{-6}$ ] per genome per  
126 generation. From the literature, the mutation rate per base pair is roughly  $2 - 3 \cdot 10^{-10}$  (refs.<sup>74,35</sup>), but  
it may be higher under heat stress, as several stresses<sup>20</sup>, including heat<sup>22</sup>, may cause hypermutation  
128 in yeast. If we assume a 10-fold increase over the mutation rate reported in the literature, then the  
estimated beneficial mutation rate can be explained by a genomic target size of 1,000 base pairs  
130 (that is, 1,000 base pairs across the genome in which a mutation would provide a fitness benefit).

Supporting this, Jarolim et al.<sup>25</sup> found 279 genes that contributed to survival after a sudden shift from  
132 30 °C to 50 °C, and Flynn et al.<sup>14</sup> used a deep mutational scan of a single protein, Hsp90, to find 465  
amino-acid variants that increased growth rate in 37 °C. Furthermore, Yona et al.<sup>70</sup> found at least 10  
134 genes on Chromosome III that increased heat tolerance when over-expressed. Assuming that other  
chromosomes also have a similar number of heat-tolerance genes (and even more, as Chromosome

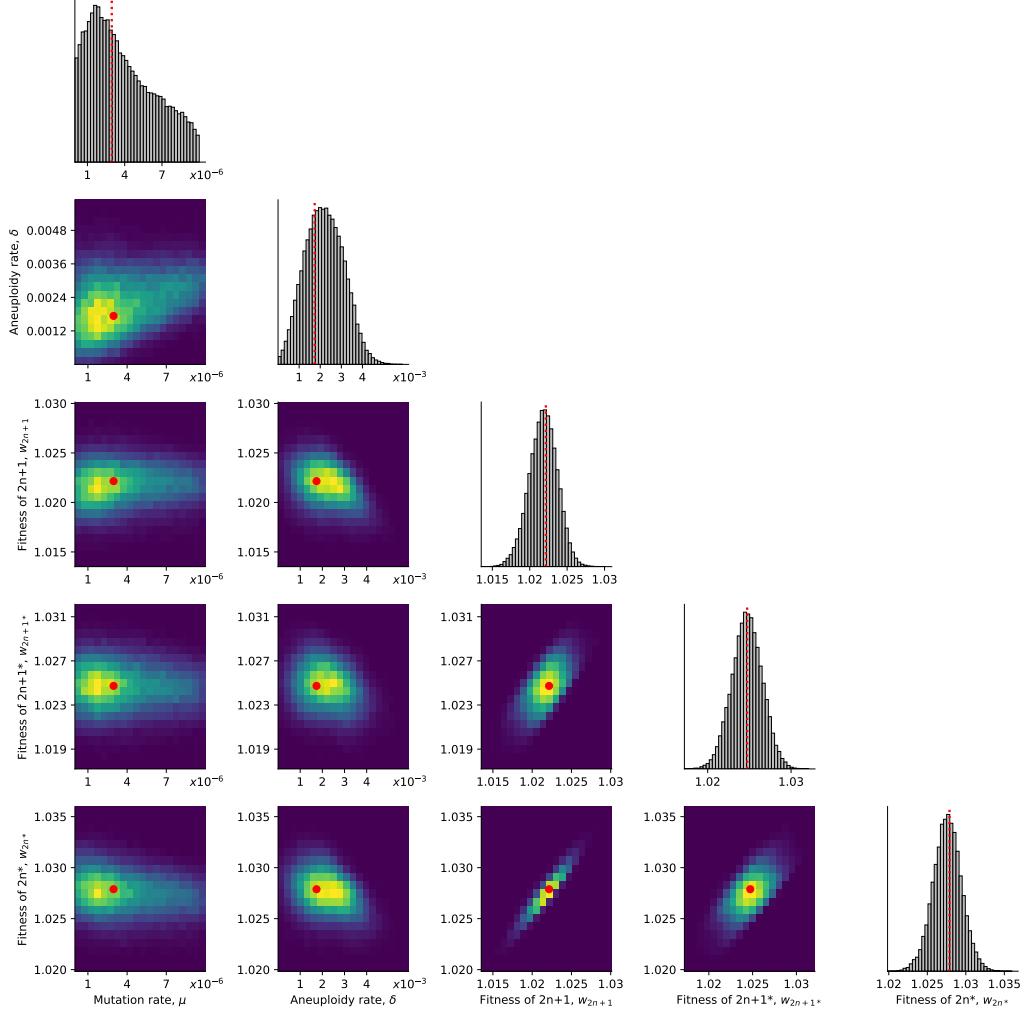


**Figure 2: Model Illustration.** There are four genotypes in our model: euploid wild-type,  $2n$ ; euploid mutant,  $2n^*$ ; aneuploid wild-type,  $2n+1$ ; and aneuploid mutant,  $2n+1^*$ . Overall there are two possible trajectories from  $2n$  to  $2n^*$ . Arrows denote transitions between genotypes, with transition rates  $\mu$  for the beneficial mutation rate and  $\delta$  for the aneuploidy rate. Elevation differences illustrate the expected, rather than the assumed, fitness differences between the genotypes.

- 136 III is one of the smallest chromosomes<sup>18</sup>), we get a total of 160 heat-tolerance genes in the genome.  
 Indeed, mutations were found in 97 genes in an evolutionary experiment with yeast under heat stress<sup>22</sup>.
- 138 Thus, to get a genomic target size of 1,000, it is enough that the average gene target size (number of  
 base pairs in a gene in which a mutation is beneficial) is 6.25 base pairs. For example, Kohn and  
 140 Anderson<sup>30</sup> found a target size of 11 in a proton exporter gene (*PMA1*) that contributes to high-salt  
 adaptation.
- 142 The estimated aneuploidy rate,  $\delta = 1.72 \cdot 10^{-3}$  [ $1.47 \cdot 10^{-3} - 2.786 \cdot 10^{-3}$ ] is higher than in previous  
 studies: for Chromosome III in diploid *S. cerevisiae*, Zhu et al.<sup>74</sup> estimated  $6.7 \cdot 10^{-6}$  chromosome  
 144 gain events per generation, and Kumaran et al.<sup>32</sup> estimate  $3.0 \cdot 10^{-5} - 4.3 \cdot 10^{-5}$  chromosome loss  
 events per generation (95% confidence interval). However, this difference may be partly explained  
 146 by an increased aneuploidy rate during heat stress: heat shock can increase the rate of chromosome  
 fragment loss by 2-3 orders of magnitude<sup>5</sup>.
- 148 The estimated fitness values are  $w_{2n+1} = 1.022$  [ $1.021 - 1.023$ ],  $w_{2n+1^*} = 1.025$  [ $1.024 - 1.026$ ],  
 $w_{2n^*} = 1.028$  [ $1.026 - 1.029$ ], all relative to the fitness of  $2n$ , which is set to  $w_{2n} = 1$ . Thus, we  
 150 can infer that the cost of Chromosome III trisomy is  $c = w_{2n^*} - w_{2n+1^*} = 0.003$  (or 0.3%) and the  
 benefit of trisomy is  $w_{2n+1} - 1 - c = 0.019$  (1.9%), whereas the benefit of the beneficial mutation is

152  $w_{2n^*} - 1 = 0.028$  (2.8%).

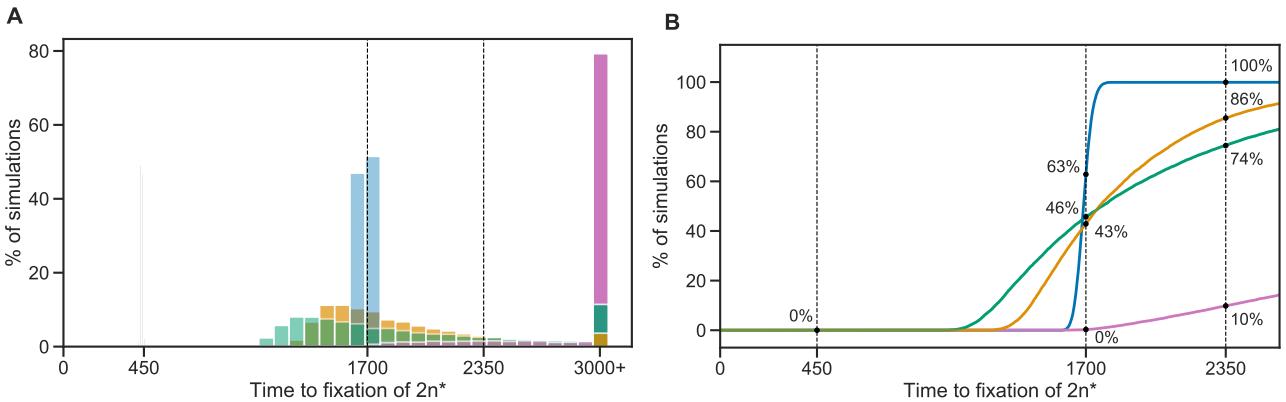
If we allow for transitions (mutation, chromosome loss and gain) to less-fit genotypes (e.g.,  $2n^*$  to  
 154  $2n+1^*$ ), then we infer similar but slightly different values, see Supplementary Material.



**Figure 3: Posterior distribution of model parameters.** On the diagonal, the marginal posterior distribution of each model parameter. Below the diagonal, the joint posterior distribution of pairs of model parameters (dark purple and bright yellow for low and high density, respectively). Red markers and orange lines for the joint MAP estimate (which may differ from the marginal MAP, as the marginal distribution integrates over all other parameters).

**Model comparison and goodness-of-fit.** To assess the fit of our model to the data, we use posterior  
 156 predictive checks, in which we simulate the frequency dynamics using MAP parameter estimates and  
 compare them to the data. Our model fits the data well:  $2n^*$  fixed in 63% of simulations by generation  
 158 1,700 and in 100% of simulations by generation 2,350 (Figure 4).

However, a model without aneuploidy (where the aneuploidy rate is fixed at zero,  $\delta = 0$ ), fails to  
 160 explain the experimental observations (Figure 4). The estimated mutation rate without aneuploidy is



**Figure 4: Model fit with and without aneuploidy.** The distribution of time to fixation of  $2n^*$  (i.e., adaptation time) in 10,000 simulations using MAP parameters of the model with beneficial aneuploidy (blue;  $\delta > 0$ ,  $w_{2n} < w_{2n+1} < w_{2n+1}^* < w_{2n}^*$ ) compared to alternative models: a model with the same parameter values but without aneuploidy (gray,  $\delta = 0$ , concentrated at  $t = 450$ ); a model fitted to the data assuming no aneuploidy (green,  $\delta = 0$ ); a model fitted to the data assuming neutral aneuploidy (yellow,  $\delta > 0$ ,  $w_{2n+1} = w_{2n}$ ,  $w_{2n+1}^* = w_{2n}^*$ ); and a model with beneficial aneuploidy and an extended prior distribution (pink). In the experiment by Yona et al.<sup>70</sup>, one population lost aneuploidy by generation 1,700 and another by generation 2,350 (dashed lines) but not before generation 450. Thus, the blue distribution has a better fit compared to the other distributions (the gray distribution has a particularly poor fit). The MAP likelihood (eq. (4)) is 0.84, 0.78, 0.67, and 0.14 for the models represented by blue, yellow, green, and pink distributions, respectively. **(A)** Histogram of the time to fixation of  $2n^*$ . The last bin contains all values equal or greater than 3,000. **(B)** Cumulative distribution of the time to fixation.

$\mu = 7.98 \cdot 10^{-9}$  [ $7.906 \cdot 10^{-9} - 8.138 \cdot 10^{-9}$ ], much lower compared to a model with aneuploidy. The 162 fitness of the mutant is also much lower at  $w_{2n}^* = 1.013$  [1.012 – 1.013]. This is because, without 164 aneuploidy, a high mutation rate or fitness effect will lead to faster appearance and fixation of  $2n^*$  than in the experimental observations.

We also checked a model in which aneuploidy occurs but is adaptively neutral compared to the wild-type, that is,  $w_{2n+1} = w_{2n}$  and  $w_{2n+1}^* = w_{2n}^*$  but  $\delta > 0$ . This model fits the data better than the model with no aneuploidy (in which  $\delta = 0$ ), but worse than a model with positive selection for aneuploidy, 168 in which  $w_{2n} < w_{2n+1} < w_{2n+1}^* < w_{2n}^*$  (Figure 4).

**Model predictions of genotype frequency dynamics.** We simulated 50 replicate genotype frequency dynamics using the MAP estimate parameters. Figure 5A shows the simulated frequencies of the four genotypes ( $2n$ ,  $2n+1$ ,  $2n+1^*$  and  $2n^*$ ), as well as the frequencies of  $2n^*$  cells that arose from either  $2n+1$  cells via a sequences of mutation and chromosome loss events ( $2n_A^*$ ), or directly from

2n cells via a mutation event ( $2n_M^*$ ). We find that  $2n+1^*$  never reaches substantial frequency as it is  
174 quickly replaced by  $2n^*$  in a process similar to *stochastic tunneling*<sup>24,31</sup>.

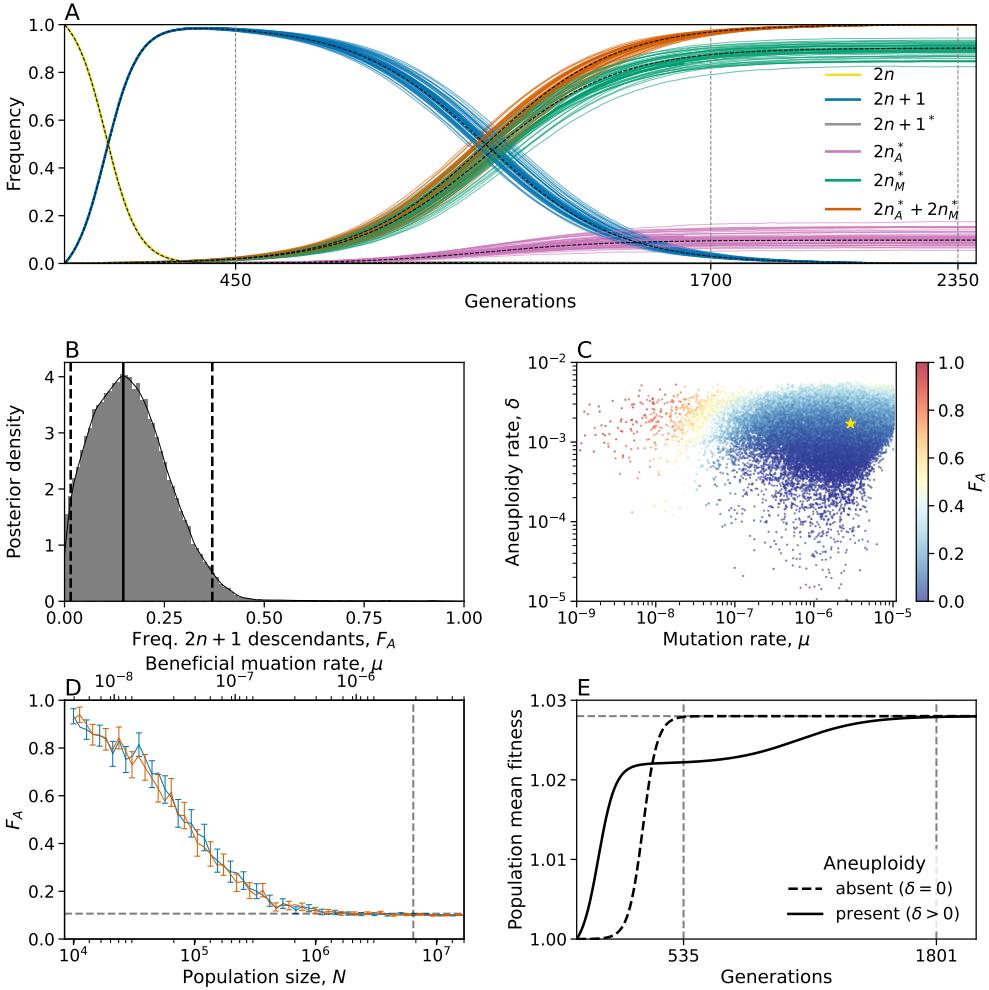
To test the hypothesis that aneuploidy facilitates adaptation, we estimated  $F_A$ , the expected frequency  
176 of  $2n^*$  that arose from  $2n+1$ , computed as the average frequency of such  $2n_A^*$  cells at the end of  
simulations using the MAP estimate parameters. Surprisingly, we observe that the majority of  $2n^*$   
178 cells are  $2n_M^*$ , a product of a direct mutation in 2n cells, rather than descending from  $2n+1$  cells  
( $F_A^{MAP} = 0.106$ , average end point of 50 purple lines in Figure 5A). This is despite the fact that the  
180  $2n+1$  genotype reaches high frequencies in the population (at least 0.98, Figure 5A).

This result is not unique to the MAP parameter estimate. We simulated genotype frequency dynamics  
182 using parameter samples from the posterior distribution, and computed the posterior distribution of  
 $F_A$  (Figure 5B). The posterior mode  $F_A$  was just 0.147 [0.0154-0.370 95% CI] and only in 489 of  
184 100,000 posterior samples (0.489%)  $F_A$  was larger than 0.5 (see Supporting Material for results when  
transitions to less-fit genotypes are allowed, such as  $2n^*$  to  $2n+1^*$ ). Thus, if we sample a random  
186 cell from the evolved  $2n^*$  population, it is more likely to have descended directly from an euploid  
cell than from an aneuploid cell. The probability of  $2n^*$  descending from  $2n+1$  ( $F_A$ ) increases  
188 with the aneuploidy rate,  $\delta$ , and decreases with both the population size  $N$  and the mutation rate,  $\mu$   
(Figure 5C,D). In some cases it can also be affected by the fitness parameters (Figure S10).

190 **Genetic instability in aneuploid cells.** It has been suggested that aneuploidy increases genetic  
instability: Sheltzer et al.<sup>56</sup> have demonstrated a fold increase of between 2.2 and 7.1 in mutation  
192 rate. Therefore, we inferred model parameters under the assumption that the mutation rate increases  
in aneuploid cells by a factor  $\tau = 1, 33/32$  (due to an additional chromosome), 2, 5, 10, or 100 (due  
194 to genetic instability). We found that the posterior distribution was similar for  $\tau = 1, 33/32, 2$ , and 5  
(Figure S4). Furthermore, we computed the WAIC, a criterion for model selection (Methods). The  
196 WAIC values were similar for all  $\tau$  values (Table S3).

Assuming a strong increase of the mutation rate in aneuploid cells, i.e.  $\tau = 100$ , the inferred a  
198 mutation rate was  $\mu = 4.094 \cdot 10^{-7}$  [ $6.252 \cdot 10^{-8} - 6.046 \cdot 10^{-7}$ ]), and the inferred aneuploidy rate  
that was  $\delta = 0.744 \cdot 10^{-3}$  [ $0.506 \cdot 10^{-3} - 1.827 \cdot 10^{-3}$ ]. Compared to inference made assuming no  
200 effect of aneuploidy on the mutation rate, these rates were about 7-8-fold and 2-3-fold lower for  $\mu$  and  
 $\delta$ , respectively. Assuming  $\tau = 10$ , the inferred a mutation rate was only slightly lower compared to  
202  $\tau = 1$  ( $\mu = 1.67 \cdot 10^{-6}$  [ $2.836 \cdot 10^{-8} - 2.245 \cdot 10^{-6}$ ]).

Therefore, we do not find any evidence of an increase in mutation rate in aneuploid cells. This may



**Figure 5: Predicted frequency of aneuploid-descended cells.** (A) Posterior predicted genotype frequencies over time, including the source of  $2n^*$ :  $2n_A^*$  arose from  $2n+1$ , whereas  $2n_M^*$  arose directly from  $2n$ . Colored curves are 50 simulations using the MAP estimate parameters. Black dashed curves are the expected genotype frequencies without genetic drift (from a deterministic model). See Figure S9 for log-log scale, in which the sequence of events is easier to observe. (B) Posterior distribution of  $F_A$ , the expected frequency of  $2n^*$  cells descended from  $2n+1$  cells, computed as the average frequency at the end of 100 simulations for 100,000 samples from the parameter posterior distribution. Solid and dashed lines show the mode and 95% CI. (C)  $F_A$  values (color coded) from panel B, with their corresponding mutation rate  $\mu$  on x-axis and aneuploidy rate  $\delta$  on the y-axis. Yellow star shows the MAP estimate. See also Figure S10. (D)  $F_A$  as a function of the population size ( $N$ , bottom x-axis) and the beneficial mutation rate ( $\mu$ , top x-axis) in posterior predictions with MAP parameters. Markers show  $F_A$  in 250 simulations per population size or mutation rate value. Error bars show mean  $F_A$  with 95% CI (bootstrap,  $n = 10,000$ ). Blue and red bars for varying population size and mutation rate, respectively. Vertical dashed line for population size in the experiment,  $6.425 \cdot 10^6$ , and the MAP mutation rate,  $2.965 \cdot 10^{-6}$ . Horizontal line for  $F_A^{MAP} = 0.106$ . (E) Population mean fitness in a model without drift using MAP estimate parameters. Solid lines for mean fitness with aneuploidy ( $\delta > 0$ ), where the population reaches adaptation (mean fitness at 99.99% of maximum value) at generation 1,802. Dashed lines for mean fitness without aneuploidy ( $\delta = 0$ ), where the population adapts much earlier, at generation 535.

204 be because, unless the increase is strong ( $\tau \geq 10$ ), it does not seem to affect our inference; or because  
205 Chromosome III is one of the smallest chromosomes<sup>18</sup>. We also checked the differences in genotype  
206 frequency dynamics for different  $\tau$  values. We observe  $\tau = 100$  could be distinguished if accurate  
207 data was available for the waiting time until the frequency of  $2n$  to decrease below 95% (Figure S5A)  
208 or for waiting time for the frequency of  $2n+1$  to either reach or go below 95% (Figure S5B). Similarly,  
209 we did not find evidence for an increase in the aneuploidy rate in aneuploid cells<sup>56</sup>, probably due to  
210 lack of statistical power.

## Discussion

212 In a study on the role of chromosome duplication in adaptive evolution, Yona et al.<sup>70</sup> found that a  
213 Chromosome III trisomy was acquired by *S. cerevisiae* populations evolving under heat stress, only  
214 to be later replaced by euploid mutant cells that carry "refined" solutions to the stress. Additionally,  
215 such a replacement also occurred when they initiated evolutionary experiments with a population in  
216 which all cells carry a Chromosome III trisomy. They hypothesized that aneuploidy is a "useful yet  
217 short-lived intermediate that facilitates further adaptation", suggesting that the euploid mutant cells  
218 evolved by heat-resistance mutations in aneuploid cells followed by reversion of trisomy due to a  
219 chromosome loss event.

220 If indeed the evolved euploid population is descended from the aneuploid population, then mutant  
221 alleles that were common in the aneuploid populations should also be common in the evolved euploid  
222 population. However, this is likely not the case (Figure 1): mutant allele frequencies in the aneuploid  
223 and euploid populations are negatively correlated, such that common alleles in the former are rare  
224 in the latter. Furthermore, we developed an evolutionary genetic model of adaptive evolution by  
225 aneuploidy and mutation (Figure 2), fitted it to the experimental results of Yona et al.<sup>70</sup>, and used  
226 it to predict the genotype frequency dynamics. The model predicted that only about 10-15% of the  
227 evolved euploid population descended from aneuploid cells by acquiring a mutation and losing the  
228 extra chromosome—that is, the majority of the euploid population are not descended from aneuploid  
229 cells, but rather are direct descendants of the ancestral wild-type population (Figure 5).

230 This happens despite aneuploidy reaching a high frequency in the population (>95%). Conventional  
231 wisdom might suggest that once the aneuploid genotype  $2n+1$  reaches high frequency, it will have a  
232 better chance at producing "refined" solutions via mutations, and its descendants will come to dominate  
233 the population: the frequency of  $2n_A^*$  (which arises from  $2n+1^*$ ) will be higher than the frequency of  
234  $2n_M^*$  (which arises directly from  $2n$ ).

So how does  $2n_M^*$  prevail? Initially, the supply rates of  $2n+1$  and  $2n_M^*$  are  $N\delta \approx 11,000$  and  $N\mu \approx 19$ , respectively (assuming MAP parameter estimates). Therefore, both genotypes are expected to appear immediately at the beginning of the experiment (Figure S9). However,  $2n+1$  appears at a much higher frequency as  $\delta \gg \mu$  by 2-3 orders of magnitude. After they first appear,  $2n_M^*$  has higher fitness. But as long as the frequency of  $2n$  is high, the supply rate of  $2n+1$  is higher than that of  $2n_M^*$ , again due to  $\delta \gg \mu$ . However, supply rates of both genotypes decreases with the frequency of  $2n$ . Therefore, when the latter decreases, mainly due to the increase in the frequency of  $2n+1$ , both supply rates diminish. At this stage, the higher fitness of  $2n_M^*$  comes into play and it starts to take over the population, which is mainly composed of  $2n+1$ . For the aneuploid lineage to compete with the mutant lineage, it must produce  $2n_A^*$  via a mutation followed by chromosome loss. Although this is a stochastic process (due to drift), our results show that the time until  $2n_A^*$  reaches a frequency of 0.1% is roughly 450 generations, without much variation (intersection of purple lines and vertical dashed line in Figure S9). However, by that time  $2n_M^*$  is already at a roughly 10-fold higher frequency (1.86%), and since both mutants have the same fitness, their relative frequency remains roughly the same until the end of the experiment.

**Predictions for small populations and low mutation rates.** We examined the effect of the population size,  $N$ , and the beneficial mutation rate,  $\mu$ , on the frequency of  $2n+1$  descendants in the evolved population,  $F_A$ . We found that  $F_A$  is expected to decrease as the population size or mutation rate increase (Figure 5D), ranging from >90% when the population size is 10,000 or the mutation rate is  $6 \cdot 10^{-9}$ , to about 10% when the population size is above 1,000,000 (less than the experimental population size, which was 6,425,000) or the mutation rate is above  $2 \cdot 10^{-6}$  (less than the inferred mutation rate, which is  $2.965 \cdot 10^{-6}$ ). Thus, our model provides a testable prediction: if the experiment was repeated under a lower population size (via stronger daily dilutions or in a smaller volume) or a lower mutation rate (via a non-mutagenic stress or stress with a smaller target size such as drug resistance), then the fraction of the population descending from aneuploid cells would be much higher.

**Aneuploidy delays rather than facilitates adaptation.** An additional interesting result of our study is that aneuploidy increases, rather than decreases, the adaptation time (Figure 5E). This happens despite the fact that the mean fitness initially increases faster in the presence of aneuploidy (Figure 5E). This is because once  $2n+1$  is common, selection for the mutant strain ( $2n+1^*$  or  $2n^*$ ) is weaker compared to when  $2n^*$  competes directly with  $2n$ .

**Rate and fitness effect of aneuploidy and mutation.** We inferred the rates of aneuploidy and mutation and their effects on fitness. We estimate that the aneuploidy rate (i.e., number of chromosome gains per generation) is  $1.7 \cdot 10^{-3}$ , higher than a previous estimate of  $6.7 \cdot 10^{-6}$  (ref 73). This may be due to genetic instability caused by heat stress<sup>5</sup>. In addition, we find no evidence for increased mutation rates in aneuploid cells. Previous empirical studies have suggested that genetic instability (e.g., elevated mutation rates) in aneuploid cells is due to stress associated with the aneuploid state<sup>3,6,71,23</sup>. However, in the experiment of Yona et al.<sup>70</sup>, both the wild-type and the aneuploid were under heat stress, which may explain why we did not find evidence for an increased mutation rate specifically in aneuploid cells.

**Conclusions.** Here, we tested the hypothesis that aneuploidy cells are an evolutionary "stepping stone", or adaptive intermediate, between wild-type euploid cells and mutant euploid cells<sup>69</sup>. Our results suggest that, although it seems the population goes from euploid to aneuploid and back, this is not the case at the individual level. We estimate that only about 10-15% of the euploid cells descended from aneuploid cells, whereas the rest are direct descendants of the wild-type euploid cells. Thus, aneuploidy can delay, rather than accelerate, adaptation, and cells that become aneuploid may leave less descendants than cells that remain euploid. This surprising result reinforces the importance of mathematical models when interpreting evolutionary dynamics. Moreover, our study emphasizes the unintuitive outcomes of clonal interference between mechanisms for generation of variation that differ in their rate of formation and distribution of fitness effects, including mutation, copy number variation, horizontal gene transfer, and epigenetic modifications.

## Models and Methods

**DNA sequencing.** Whole-genome sequencing of the ancestral diploid strain ( $2n$ ) was performed on a single colony of the ancestor. Whole-genome sequencing of the four evolving populations ( $H2$  after 450 and 2,350 generations, and  $H4$  after 450 and 1,700 generations) was performed on a sample from these populations (rather than from single colonies) in order to maintain the population diversity. Cells were grown in 5ml of YPD medium, either at  $30^{\circ}\text{C}$  (ancestral diploid) or  $39^{\circ}\text{C}$  (evolved populations) in shaking conditions (200rpm) until reaching stationary phase. Following growth, 3ml of each culture were centrifuge (14,000rpm) and cell pellets were used for DNA extraction. Genomic DNA was extracted using "MasterPure Yeast DNA Purification Kit" (Lucigen) according to the manufacture instructions. Following extraction, DNA concentrations were determined by Qubit assay (Thermo Fisher) and  $\sim 1\mu\text{g}$  DNA was used for library preparation using Illumina sample

296 preparation kit (Illumina). Samples were sequenced using a 100 bp pair end read output run using  
Illumina HiSeq2500.

298 **Evolutionary genetic model.** We model the evolution of a population of cells using a Wright-Fisher model<sup>40</sup>, assuming a constant effective population size  $N$ , non-overlapping generations, and  
300 including the effects of natural selection, genetic drift, aneuploidy, and mutation. We focus on beneficial  
factors genetic modifications, neglecting the effects of deleterious and neutral mutations or karyotypic  
302 changes. The model allows for a single aneuploid karyotype (e.g., Chromosome III duplication) and  
a single mutation to accumulate in the genotype. Thus, the model follows four genotypes (Figure 2):  
304 euploid wild-type,  $2n$ , the initial genotype; euploid mutant,  $2n^*$ , with the standard karyotype and  
a single beneficial mutation; aneuploid wild-type,  $2n+1$ , with an extra chromosome, i.e., following  
306 chromosome duplication; and aneuploid mutant,  $2n+1^*$ , with an extra chromosome and a beneficial  
mutation.

308 Transitions between the genotypes occur as follows (Figure 2): Beneficial mutations from  $2n$  to  $2n^*$   
and from  $2n+1$  to  $2n+1^*$  occur with probability  $\mu$ , the mutation rate. We neglect back-mutations (i.e.,  
310 from  $2n^*$  to  $2n$  and from  $2n+1^*$  to  $2n+1$ ). Aneuploidy is formed by chromosome mis-segregation,  
so that cells transition from  $2n$  to  $2n+1$  and from  $2n+1^*$  to  $2n^*$  with probability  $\delta$ , the aneuploidy  
312 rate. That is, we assume chromosomes are gained and lost at the same rate, and we neglect events  
that form a less-fit genotype (i.e.,  $2n+1$  to  $2n$  and  $2n^*$  to  $2n+1^*$ ). A model that assumed increased  
314 aneuploidy rates in aneuploid cells (as in Sheltzer et al.<sup>56</sup>) did not perform well, probably due to lack  
of statistical power, and was abandoned.

316 In the experiment by Yona et al.<sup>70</sup>, the population was grown every day from  $1.6 \cdot 10^6$  cells until  
reaching stationary phase and then diluted 1:120. Thus, we set the population size to  $N = 6.425 \cdot 10^6$ ,  
318 the harmonic mean of  $\{2^k \cdot 1.6 \cdot 10^6\}_{k=0}^7$ <sup>10</sup>. The initial population has  $N$  cells with genotype  $2n$ .  
The effect of natural selection on the frequency  $f_i$  of genotype  $i = 2n, 2n + 1, 2n + 1^*,$  or  $2n^*$  is given  
320 by

$$f_i^s = \frac{f_i w_i}{\bar{w}}, \quad (1)$$

322 where  $w_i$  is the fitness of genotype  $i$  and  $\bar{w} = \sum_j f_j w_j$  is the population mean fitness. The effect of

mutation and aneuploidy on genotype frequencies is given by

$$\begin{aligned}
 f_{2n}^m &= (1 - \delta - \mu) f_{2n}^s, \\
 f_{2n+1}^m &= \delta f_{2n}^s + (1 - \mu) f_{2n+1}^s, \\
 f_{2n+1^*}^m &= \mu f_{2n+1}^s + (1 - \delta) f_{2n+1^*}^s, \\
 f_{2n^*}^m &= \mu f_{2n}^s + \delta f_{2n+1}^s + f_{2n^*}^s.
 \end{aligned} \tag{2}$$

Finally, random genetic drift is modeled using a multinomial distribution<sup>40</sup>,

$$\mathbf{f}' \sim \frac{1}{N} \cdot \text{Mult}(N, \mathbf{f}^m), \tag{3}$$

where  $\mathbf{f}^m = (f_{2n}^m, f_{2n+1}^m, f_{2n+1^*}^m, f_{2n^*}^m)$  are the frequencies of the genotypes after mutation and aneuploidy,  $\mathbf{f}'$  are the genotype frequencies in the next generation, and  $\text{Mult}(N, \mathbf{f})$  is a multinomial distribution parameterized by the population size  $N$  and the genotype frequencies  $\mathbf{f}$ . Overall, the change in genotype frequencies from one generation to the next is given by the transformation  $f_i \rightarrow f'_i$ .

**Empirical data for model inference.** We use the results of evolutionary experiments reported by

Yona et al.<sup>70</sup>. In their heat-stress experiment, four populations of *S. cerevisiae* evolved under 39 °C. Aneuploidy fixed in all four population in the first 450 generations. Hereafter, fixation or elimination of a genotype *by generation t* means that more than 95% or less than 5% of the population carry the genotype at generation  $t$ , and possibly earlier. From re-analysis of data not published in the original paper, aneuploidy did not fix before at least 200 generations elapsed. The experiment continued with two populations, in which aneuploidy was eliminated by generation 1,700 and 2,350 while still under the same conditions of elevated heat (39 °C).

**Likelihood function.** Because our model, just like the Wright-Fisher model, is non-linear and

stochastic, computing the distribution of fixation time  $T(g)$  of genotype  $g$  for use in the likelihood function is intractable (it is even hard to use a diffusion-equation approximation due to the model having multiple genotypes, rather than just two). We overcome this problem by approximating the likelihood using simulations. We simulate 1,000 experiments per parameter vector  $\theta = (\mu, \delta, s, b, c)$ , resulting in a set of simulated observations  $\tilde{\mathbf{X}} = \{\tilde{X}_i\}_{i=1}^{1000}$ . We then compute the approximate likelihood,

$$\begin{aligned}
 \mathcal{L}(\theta) = P^4(200 \leq T(2n+1) \leq 450) \cdot & \left[ 1 - \right. \\
 & P_{\tilde{\mathbf{X}}}^4(\{T(2n^*) < 1700\} \mid 200 \leq T(2n+1) \leq 450) - \\
 & P_{\tilde{\mathbf{X}}}^4(\{1700 < T(2n^*) < 2350\} \mid 200 \leq T(2n+1) \leq 450) + \\
 & \left. P_{\tilde{\mathbf{X}}}^4(\{T(2n^*) < 1700\} \wedge \{1700 < T(2n^*) < 2350\} \mid 200 \leq T(2n+1) \leq 450) \right], \tag{4}
 \end{aligned}$$

346 where  $!\{\dots\}$  is the "logical not" operator,  $P^4(\dots)$  is the 4th power of  $P(\dots)$ , and all probabilities  
 $P_{\tilde{\mathbf{X}}}(\dots)$  are approximated from the results of the simulations  $\tilde{\mathbf{X}}$ . For example,  $P_{\tilde{\mathbf{X}}}(!\{T(2n^*) < 1700\} |$   
348  $200 \leq T(2n+1) \leq 450)$  is approximated by taking simulations in which  $2n+1$  fixed before generation  
450 but not before generation 200, and computing the fraction of such simulations in which  $2n^*$  did  
350 not fix by generation 1,700, and hence aneuploidy did not extinct before generation 1,700. Figure S1  
compares results with less and more simulated experiments, demonstrating that 1,000 simulations are  
352 likely sufficient.

For a model without aneuploidy (that is, when the aneuploidy rate is fixed at zero,  $\delta = 0$ ), we disregard  
354 the increased expression in Chromosome III and the growth advantage measured in generation 450,  
and focus on the growth advantage measured in later generations, presumably due to a beneficial  
356 mutation. Therefore, the likelihood is approximated by

$$\begin{aligned}\mathcal{L}_!(\theta) = 1 - P_{\tilde{\mathbf{X}}}^4(!\{T(2n^*) < 1700\}) - \\ P_{\tilde{\mathbf{X}}}^4(!\{1700 < T(2n^*) < 2350\}) + \\ P_{\tilde{\mathbf{X}}}^4(!\{T(2n^*) < 1700\} \wedge !\{1700 < T(2n^*) < 2350\}).\end{aligned}\quad (5)$$

358 **Parameter inference.** To infer model parameters, we use approximate Bayesian computation with  
a sequential Monte-Carlo scheme, or ABC-SMC<sup>59</sup>, implemented in the pyABC Python package<sup>29</sup>  
360 [pyabc.readthedocs.io](https://pyabc.readthedocs.io). This approach uses numerical stochastic simulations of the model to infer  
a posterior distribution over the model parameters. It is a method of likelihood-free, simulation-  
362 based inference<sup>9</sup>, that is, for estimating a posterior distribution when a likelihood function cannot be  
directly computed. It is therefore suitable in our case, in which the likelihood function can only be  
364 approximated from simulations, and cannot be directly computed.

The ABC-SMC algorithm employs sequential importance sampling over multiple iterations<sup>63,28,61</sup>. In  
366 iteration  $t$  of the algorithm, a set of parameter vectors,  $\{\theta_{i,t}\}_{i=1}^{n_t}$ , also called *particles*, are constructed  
in the following way. A proposal particle,  $\theta^*$ , is sampled from a proposal distribution, and is either  
368 accepted or rejected, until  $n_t$  particles are accepted. The number of particles,  $n_t$ , is adapted at every  
iteration  $t$  using the adaptive population strategy<sup>29</sup> [pyabc.readthedocs.io](https://pyabc.readthedocs.io). For  $t = 0$ , the proposal  
370 particle is sampled from the prior distribution,  $p(\theta)$ . For  $t > 0$ , the proposal particle is sampled from  
the particles accepted in the previous iteration,  $\{\theta_{i,t-1}\}_{i=1}^{n_{t-1}}$ , each with a probability relative to its weight  
372  $W_{t-1}(\theta_{i,t-1})$  (see below). The proposal particle is then perturbed using a kernel perturbation kernel,  
 $K_t(\theta^* | \theta)$  where  $\theta$  is the sample from the previous iteration. Then, a set of synthetic observations  
374  $\tilde{\mathbf{X}}^*$  is simulated, and the proposal particle  $\theta^*$  is accepted if its approximate likelihood (eq. (4)) is high  
enough,  $\mathcal{L}(\theta^*) > 1 - \epsilon_t$  (or more commonly, if  $1 - \mathcal{L}(\theta^*) < \epsilon_t$ ), where  $\epsilon_t > 0$  is the *acceptance*

376 *threshold*, as higher values of  $\epsilon_t$  allow more particles to be accepted. The acceptance threshold  $\epsilon_t$  is  
chosen as the median of the  $1 - \mathcal{L}(\theta)$  of the particles accepted in the previous iteration,  $t - 1$ , and  
378  $\epsilon_0 = 0.01$ . For each accepted particle  $\theta_{i,t}$  a weight  $W_t(\theta_{i,t})$  is assigned: for  $t = 0$ ,  $W_0(\theta_{i,0}) = 1$ ,  
and for  $t > 0$ ,  $W_t(\theta_{i,t}) = p(\theta_{i,t}) / \sum_{i=1}^{n_{t-1}} W_{t-1}(\theta_{i,t-1}) K_t(\theta_{i,t}, \theta_{i,t-1})$ , where  $p(\theta)$  is the prior density of  
380  $\theta$  and  $K_t(\theta', \theta)$  is the probability of a perturbation from  $\theta$  to  $\theta'$ .  $K_t(\theta' | \theta)$  is a multivariate normal  
distribution, fitted at iteration  $t$  to the particles from the previous iteration,  $\{\theta_{i,t-1}\}_{i=1}^{n_{t-1}}$ , and their  
382 weights,  $\{W(\theta_{i,t-1})\}_{i=1}^{n_{t-1}}$ .

Acceptance is determined according to the approximate likelihood (eq. (4)), which has a maximum  
384 value of  $\mathcal{L}_{max} = 0.875$  (giving a minimal value of  $\epsilon_{min} = 0.125$ ). We terminated the inference  
iterations when the change in  $\epsilon$  value from one iteration to the next was small. With our standard prior  
386 and model, we reached  $\epsilon = 0.13$  (or  $\mathcal{L} = 0.87$ ) after six iterations, with  $n_6 = 982$  accepted parameter  
vectors and effective sample size ESS=651 (Figure S2). Running the inference algorithm with different  
388 initialization seeds and less or more simulations for approximating the likelihood produced similar  
posterior distributions (Figure S1).

390 After producing a set of weighted particles from the the posterior distribution using the above ABC-  
SMC algorithm, we approximate the posterior using kernel density estimation (KDE) with Gaussian  
392 kernels. We truncate the estimated posterior to avoid positive posterior density for values with zero  
prior density. The MAP (maximum a posteriori) estimate is computed as the the maximum of the  
394 estimated joint posterior density. We then draw 5,000,000 samples from the posterior distribution  
to compute the HDI (highest density interval) and draw 50,000 samples to visualize the posterior  
396 distribution with histograms.

**Model comparison.** We examine several versions of our evolutionary models, e.g. without aneu-  
398 ploidy or with increased mutation rate in aneuploid cells, as well as several different prior distributions  
(see below). To compare these, we plot posterior predictions: for each model we execute 10,000  
400 simulations using the MAP parameter estimates and plot the distributions of time to fixation of  $2n^*$ ,  
one of key properties of the model likelihood. These plots visualize the fit of each model to the  
402 data. Also, for similar models we plot the marginal and joint posterior distributions of the parameters;  
if these are similar, we consider the models interchangeable. We validate this by comparing HDI  
404 (highest density interval) of posterior distributions.

Where posterior plots are very similar and the number of parameters is the same, we use WAIC, or

406 the widely applicable information criterion<sup>15</sup>, defined as

$$WAIC(\theta) = -2 \log \mathbb{E}[\mathcal{L}(\theta)] + 2\mathbb{V}[\log \mathcal{L}(\theta)] \quad (6)$$

408 where  $\theta$  is a parameter vector, and  $\mathbb{E}[\cdot]$  and  $\mathbb{V}[\cdot]$  are the expectation and variance taken over the  
409 posterior distribution, which in practice are approximated using 50,000 samples from the posterior  
410 KDE. We validated that upon resampling WAIC values do not significantly change and that differences  
411 in WAIC between models are preserved. WAIC values are scaled as a deviance measure: lower values  
412 imply higher predictive accuracy.

**Prior distributions.** We used informative prior distributions for  $w_{2n+1} = 1 - c + b$ ,  $w_{2n+1*} = (1 +$   
414  $s)(1 - c) + b$  and  $w_{2n*} = 1 + s$ , which we estimated from growth curves data from mono-culture growth  
415 experiments previously reported by Yona et al.<sup>70</sup>, Figs. 3C, 4A, and S2. We used Curveball, a method  
416 for predicting results of competition experiments from growth curve data<sup>43</sup> [curveball.yoavram.com](http://curveball.yoavram.com).  
417 Briefly, Curveball takes growth curves of two strains growing separately in mono-culture and predicts  
418 how they would grow in a mixed culture, that is, it predicts the results of a competition assay. From these  
419 predictions, relative fitness values can be computed. Because Curveball uses a maximum-likelihood  
420 approach to estimate model parameters, we were able to estimate a distribution of relative fitness  
421 values to be used as a prior distribution by sampling 10,000 samples from a truncated multivariate  
422 normal distribution defined by the maximum-likelihood covariance matrix (Figure S3).

We used growth curves of  $2n$  and  $2n+1$  in 39 °C to estimate an informative prior distribution for  
423  $w_{2n+1}$  (Figure S3-D, assuming  $w_{2n} = 1$ ). In this prior distribution, we used the same prior for  $w_{2n+1*}$   
424 and  $w_{2n*}$ . To increase computational efficiency, we also assumed  $w_{2n*} > w_{2n+1*} > w_{2n+1} > w_{2n}$ ;  
425 running the inference without this assumption produced similar results. See *supporting material* for  
426 an extended informative prior distribution that uses growth curves of  $2n*$  and  $2n+1$  growing in 39 °C;  
427 this prior distribution proved to be less useful.

As a control, we tested an uninformative uniform prior with  $U(1, 6)$ , for (i) all  $w_{2n+1}$ ,  $w_{2n+1*}$ ,  $w_{2n*}$ ,  
430 or (ii) only for  $w_{2n+1*}$ ,  $w_{2n*}$ , using the above informative prior for  $w_{2n+1}$ . In these cases the inference  
431 algorithm failed to converge.

432 For the mutation rate,  $\mu$ , and aneuploidy rate,  $\delta$ , we used uninformative uniform priors,  $\mu \sim$   
433  $U(10^{-9}, 10^{-5})$  and  $\delta \sim U(10^{-6}, 10^{-2})$ . A wider mutation rate prior,  $\mu \sim U(10^{-9}, 10^{-3})$ , produced  
434 similar results.

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# Supplementary Material

## 634 Supplementary Analysis

**Sensitivity analysis.** Changing a single parameter while keeping the rest fixed at the MAP estimate produces a worse fit to the data (Figure S6). Furthermore, we fitted models with a mutation rate fixed at  $\mu = 10^{-5}$ ,  $10^{-6}$  and  $10^{-7}$ . We inferred similar parameters estimates for the model with  $\mu = 10^{-6}$  compared to the model with a free  $\mu$  parameter, in which the inferred mutation rate is  $\mu \approx 3 \cdot 10^{-6}$ . Inference assuming  $\mu = 10^{-5}$  or  $\mu = 10^{-7}$  produced similar estimates except that the estimated aneuploidy rate,  $\delta$ , was higher, and assuming  $\mu = 10^{-7}$ , the estimated fitness of  $2n+1$  was lower (Figure S7).

642 **Extended informative prior distribution.** In an extended informative prior distribution, we used additional growth curves of  $2n^*$  (*refined* strain from Yona et al.<sup>70</sup>) and  $2n+1$  in 39 °C to estimate  $w_{2n^*}/w_{2n+1}$  (Figure S3L). The same distribution was used for  $w_{2n^*}/w_{2n+1*}$ . Thus, our main informative prior uses a single prior distribution for fitness values of  $2n+1$ ,  $2n+1^*$ , and  $2n^*$ , whereas the extended informative prior uses one distribution for  $2n+1$ , and another distribution for both  $2n+1^*$  and  $2n^*$ .

648 We estimated the parameters under this extended informative prior. Inference took much longer to run but the posterior distribution seemed to converge, as it did not change much in the final iterations. The posterior predictive plot shows that inference with this extended prior produces a posterior distribution that fails to explain the empirical observations (pink in Figure 4). However, 652 the inferred posterior distribution is considerably narrower (compare Figures 3 and S8) and therefore parameter estimates are less variable. The estimated mutation rate was much lower compared to 654 the main informative prior, with  $\mu = 2.474 \cdot 10^{-9}$  [ $2.423 \cdot 10^{-9} - 2.612 \cdot 10^{-9}$ ]. Other parameter estimates are:  $\delta = 2.705 \cdot 10^{-3}$  [ $2.094 \cdot 10^{-3} - 3.094 \cdot 10^{-3}$ ],  $w_{2n+1} = 1.022$  [ $1.021 - 1.024$ ], 656  $w_{2n+1*} = 1.052$  [ $1.05 - 1.054$ ],  $w_{2n^*} = 1.053$  [ $1.051 - 1.055$ ], the latter two being much higher compare to the main informative prior. Notably, the mode of the posterior ratio  $w_{2n^*}/w_{2n+1} = 1.0009$  658 is much lower than the mode of the prior ratio of 1.033 (Figure S3H) and closer to the ratio of 1 that we assume in the main informative prior. Together with the posterior predictive results, we conclude 660 that the main informative prior is preferable over the extended informative prior.

**Model with transitions to less-fit genotypes** We also estimated the parameters of a version of the 662 model that includes transitions (mutation, chromosome loss and gain) to less-fit genotypes (e.g.,  $2n^*$

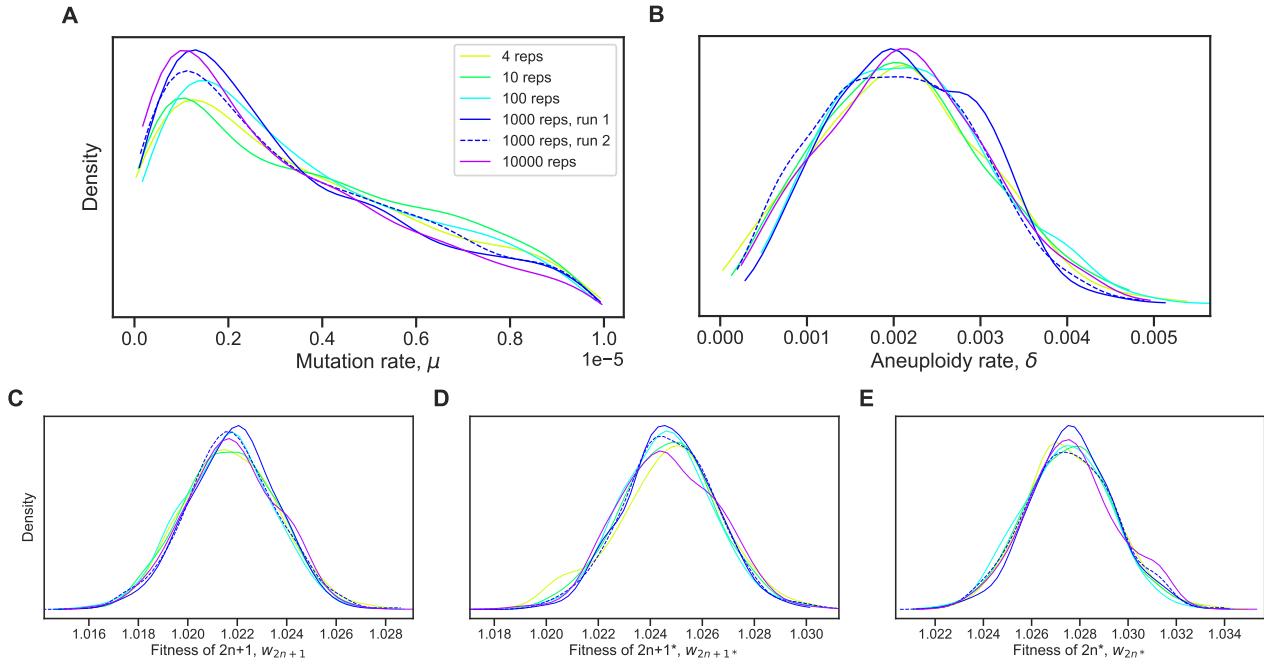
to  $2n+1^*$ ),

$$\begin{aligned}
 f_{2n}^m &= (1 - \delta - \mu)f_{2n}^s + \delta f_{2n+1}^s + \mu f_{2n^*}^s , \\
 f_{2n+1}^m &= \delta f_{2n}^s + (1 - \delta - \mu)f_{2n+1}^s + \mu f_{2n+1^*}^s , \\
 f_{2n+1^*}^m &= \mu f_{2n+1}^s + (1 - \delta - \mu)f_{2n+1^*}^s + \delta f_{2n^*}^s , \\
 f_{2n^*}^m &= \mu f_{2n}^s + \delta f_{2n+1^*}^s + (1 - \delta - \mu)f_{2n^*}^s .
 \end{aligned} \tag{7}$$

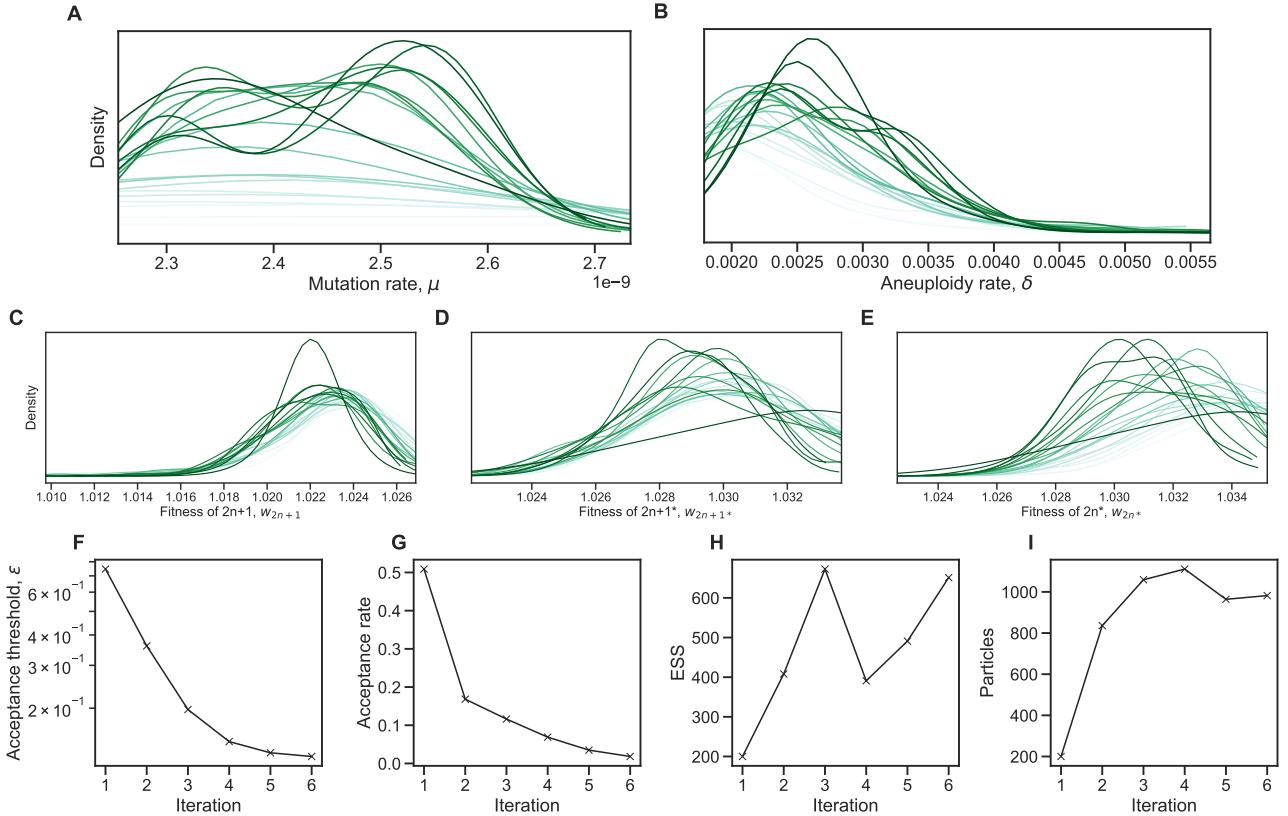
The inferred values are slightly different. The estimated mutation rate,  $\mu = 1.036 \cdot 10^{-7}$  [8.01 · 664  $10^{-8} - 1.339 \cdot 10^{-7}$ ], corresponds to a mutation target size of  $\sim 300 - 500$ , assuming the mutation rate per base pair is roughly  $2 \cdot 10^{-10}$  (ref.<sup>74</sup>) or  $3.3 \cdot 10^{-10}$  (ref.<sup>35</sup>). The estimated aneuploidy 668 rate,  $\delta = 2.358 \cdot 10^{-4}$  [ $1.766 \cdot 10^{-4} - 2.837 \cdot 10^{-4}$ ] is 5-35-fold higher than in previous studies: 670 for Chromosome III in diploid *S. cerevisiae*, Zhu et al.<sup>74</sup> estimated  $6.7 \cdot 10^{-6}$  chromosome gain 672 events per generation, and Kumaran et al.<sup>32</sup> estimate  $3.0 - 4.3 \cdot 10^{-5}$  chromosome loss events per 674 generation (95% confidence interval). The estimated fitness values are  $w_{2n+1} = 1.024$  [1.023 – 1.025], 676  $w_{2n+1^*} = 1.025$  [1.024 – 1.026],  $w_{2n^*} = 1.032$  [1.031 – 1.033], all relative to the fitness of  $2n$ , which 678 is set to  $w_{2n} = 1$ . Thus, we can infer that the cost of trisomy is  $c = w_{2n^*} - w_{2n+1^*} = 0.007$  (or 0.7%) 680 and the benefit of trisomy is  $w_{2n+1} - 1 - c = 0.017$  (1.7%), whereas the benefit of beneficial mutation is  $w_{2n^*} - 1 = 0.032$  (3.2%).

676 We simulated genotype frequency dynamics using parameter samples from the posterior distribution, 678 and computed the posterior distribution of  $F_A$ . The mean  $F_A$  in this case is just 0.0189 [0.0004 - 680 0.1214 95% CI], lower than without the transitions to less-fit genotypes. Here,  $F_A$  is the sum of frequencies of both  $2n_A^*$  and  $2n + 1_A^*$ , which reaches a frequency of 0.0007. Out of 100,000 posterior

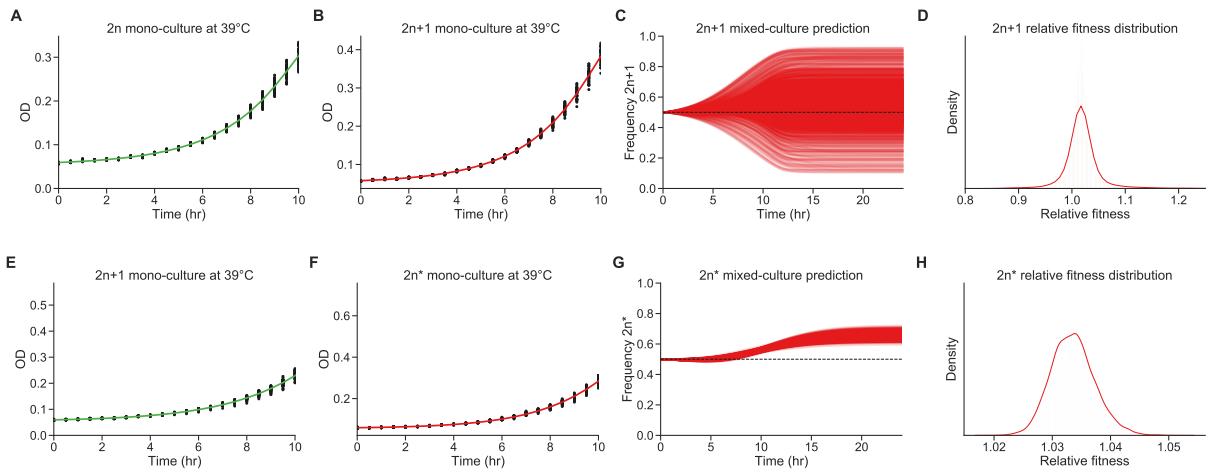
## Supplementary Figures & Tables



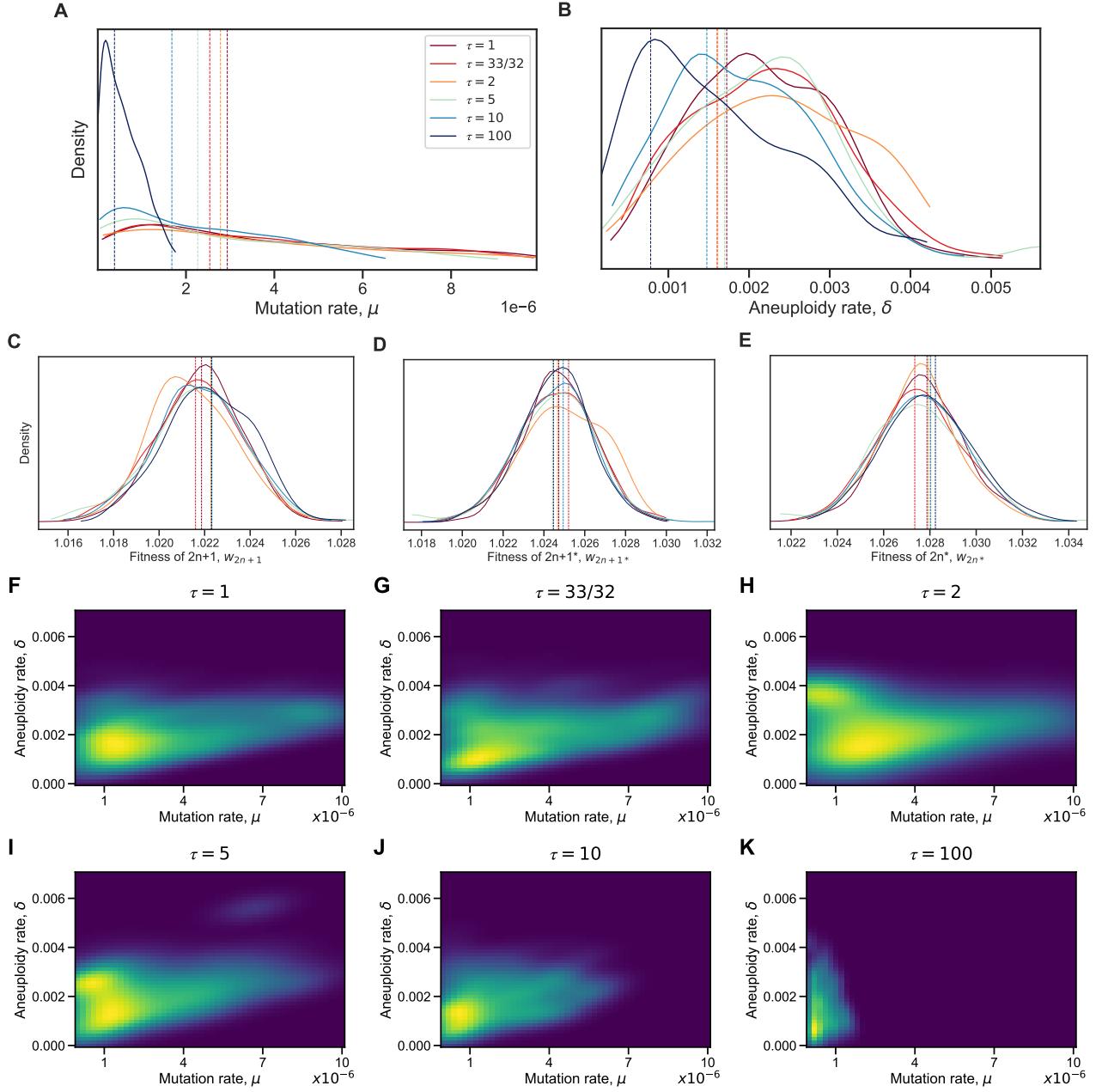
**Figure S1: Posterior distribution validation.** The posterior distribution of model parameters is roughly the same regardless of the number of simulations (4-10,000 replicates) used to approximate the likelihood (eq. (4)).



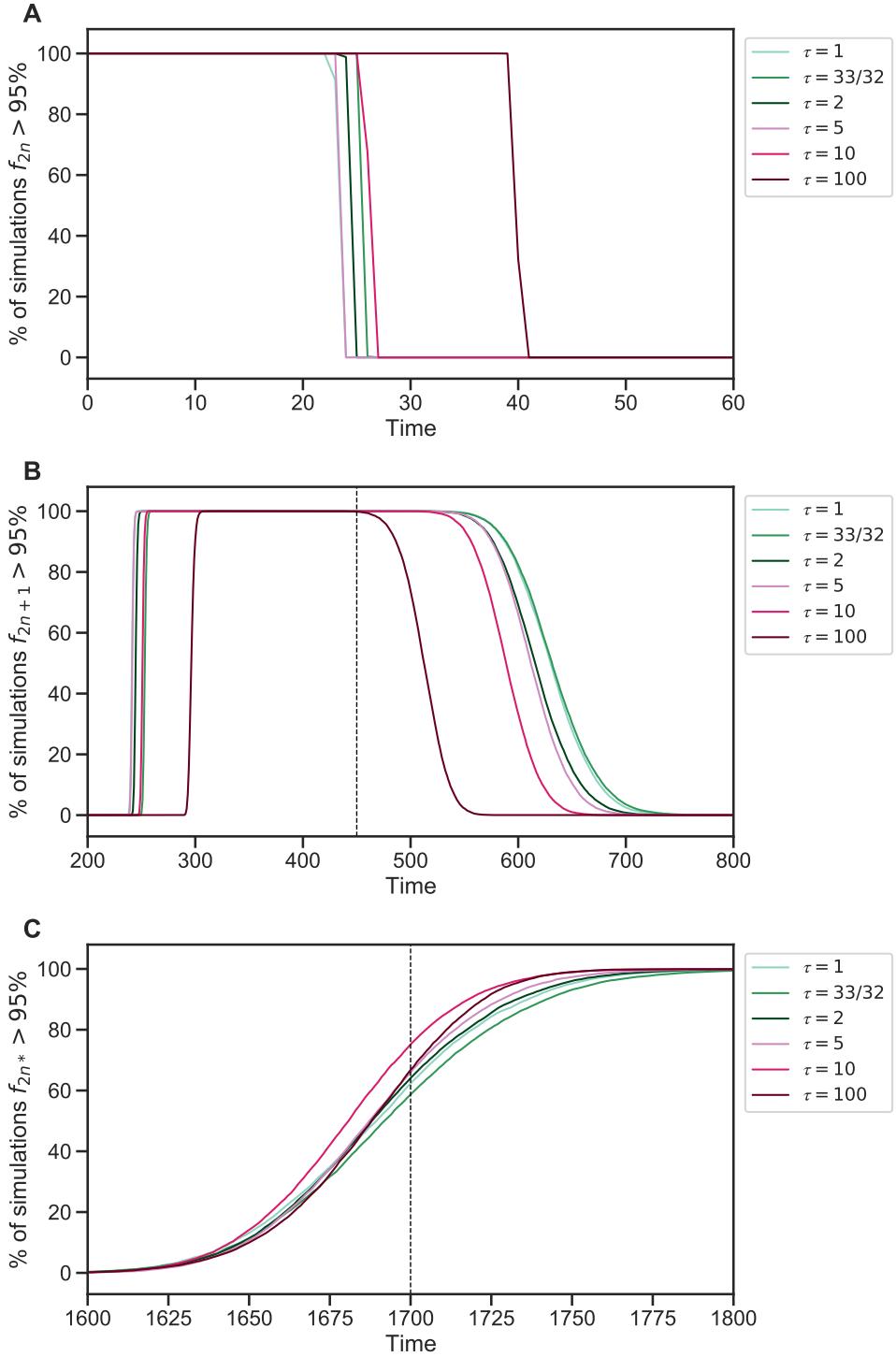
**Figure S2: Inference convergence.** The ABC-SMC algorithm was used to infer the model parameters. **(A-E)** The approximate posterior distributions of model parameters at each iteration of the ABC-SMC algorithm demonstrates convergence, as the posterior did not significantly change after the first iteration,  $t = 1$ . **(F-I)** ABC-SMC measures of convergence. After iteration number 6, the acceptance threshold was  $\epsilon = 0.13$  (i.e.,  $\mathcal{L} = 0.87$ , eq. (4)), the acceptance rate was 0.018, the number of particles was 982, and the effective sample size ESS=651.



**Figure S3: Fitness estimation from growth curves.** **(A-D)** Fitness estimation from growth curves of  $2n$  and  $2n+1$  at  $39^{\circ}\text{C}$ .  $w_{2n+1}/w_{2n}=1.024$  (95% CI: 0.959 - 1.115). **Curveball (E-H)** Fitness estimation from growth curves of  $2n+1$  and  $2n^*$  at  $39^{\circ}\text{C}$ .  $w_{2n^*}/w_{2n+1}=1.033$  (95% CI: 1.027 - 1.041). Growth curves previously described in Yona et al.<sup>70</sup>, Figs. 3C, 4A, and S2. Fitness estimated from growth curves using Curveball, a method for predicting results of competition experiments from growth curve data<sup>43</sup> [curveball.yoavram.com](http://curveball.yoavram.com). See *Models and Methods, Prior distributions* for more details. **(A,B;E,F)** Mono-culture growth curve data (markers) and best-fit growth models (lines). **(C,G)** The mixed-culture prediction for the strains from A,B and E,F respectively, 6,375 generated curves. **(D,H)** The relative fitness distribution for  $2n+1$  relative to  $2n$  (panel D) and  $2n^*$  relative to  $2n+1$  (panel H). Figures generated by Curveball.



**Figure S4: Model with elevated mutation rate in aneuploid cells.** (A-E) The inferred posterior distributions for models with different values of  $\tau$ , the fold-increase in mutation rate in aneuploid cells ( $2n+1$  and  $2n+1^*$ ). Vertical dashed lines represent the MAP (maximum a posteriori) of each distribution. When the increase in mutation rate is high,  $\tau = 10$  and  $\tau = 100$ , the inferred mutation (A) and aneuploidy (B) rates tend to be lower. (F-K) The inferred joint posterior distribution of mutation rate ( $\mu$ ) and aneuploidy rate ( $\delta$ ) with different  $\tau$  values (dark purple and bright yellow for low and high density, respectively).



**Figure S5: Genotype fixations for models with increased genetic instability.** We estimated the parameters for different models, each assuming a different value of  $\tau$ , the fold-increase in mutation rate in aneuploid cells. We then generated 10,000 simulations using the MAP estimate of each model and evaluated the fraction of simulations in which the frequency of genotype  $2n$  (**A**),  $2n+1$  (**B**), and  $2n^*$  (**C**) is above 95% (y-axis) at each generation (x-axis). Note that  $2n+1^*$  did not fix. We can see that  $\tau = 100$  can be distinguished if the waiting time for  $f_{2n} < 95\%$  is known (panel A) or if the waiting time for  $f_{2n+1} > 95\%$  or  $f_{2n+1} < 95\%$  is known (panel B). It is harder to distinguish between  $1 \leq \tau \leq 10$ .

**Table S1: Mutant alleles in population  $H2$ .**

Mutant alleles identified in the ancestor (generation 0), aneuploid (generation 450), and evolved (generation 2,350) of population  $H2$ . See supplementary file.

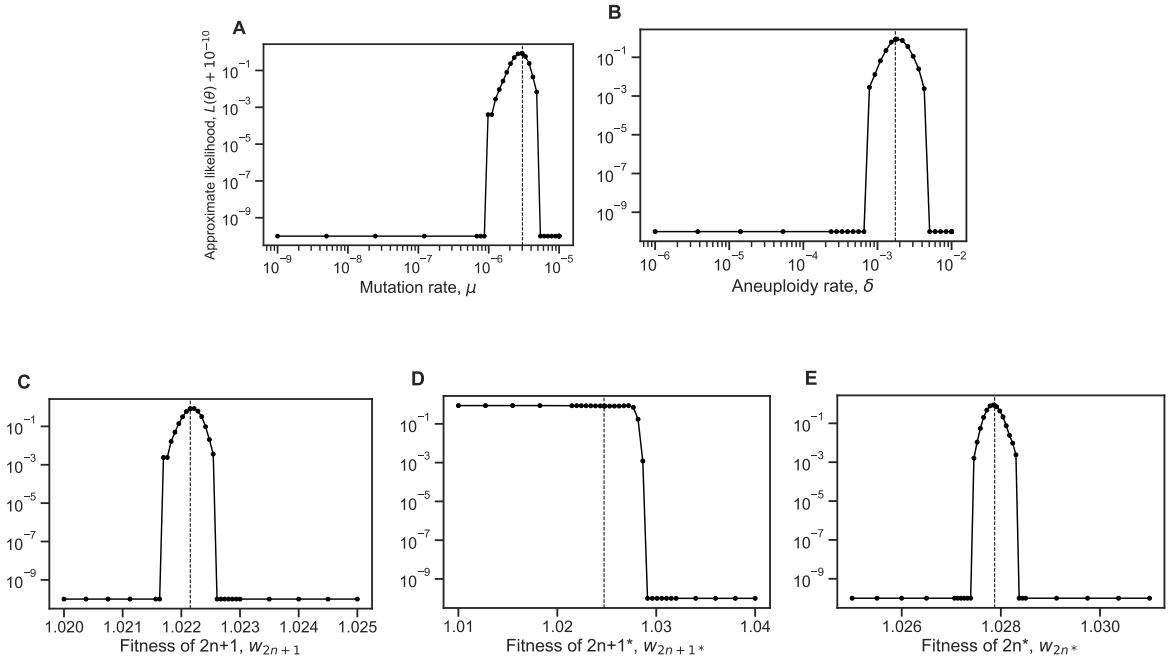
**Table S2: Mutant alleles in population  $H4$ .**

Mutant alleles identified in the ancestor (generation 0), aneuploid (generation 450), and evolved (generation 1,700) of population  $H4$ . See supplementary file.

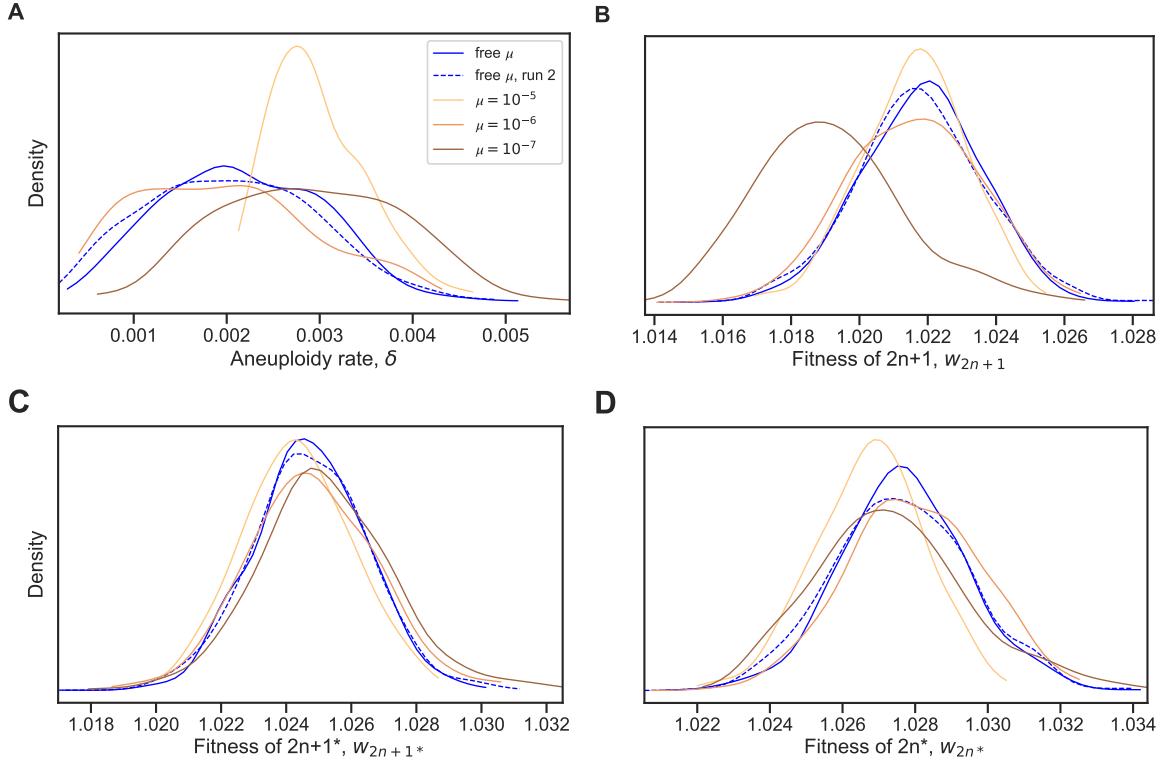
**Table S3: WAIC values for different  $\tau$  values.** Differences of less than 6 are considered of weak significance<sup>26</sup>.

Model	WAIC
$\tau = 1$	-9
$\tau = 33/32$	-9
$\tau = 2$	-8
$\tau = 5$	-12
$\tau = 10$	-9
$\tau = 100$	-12

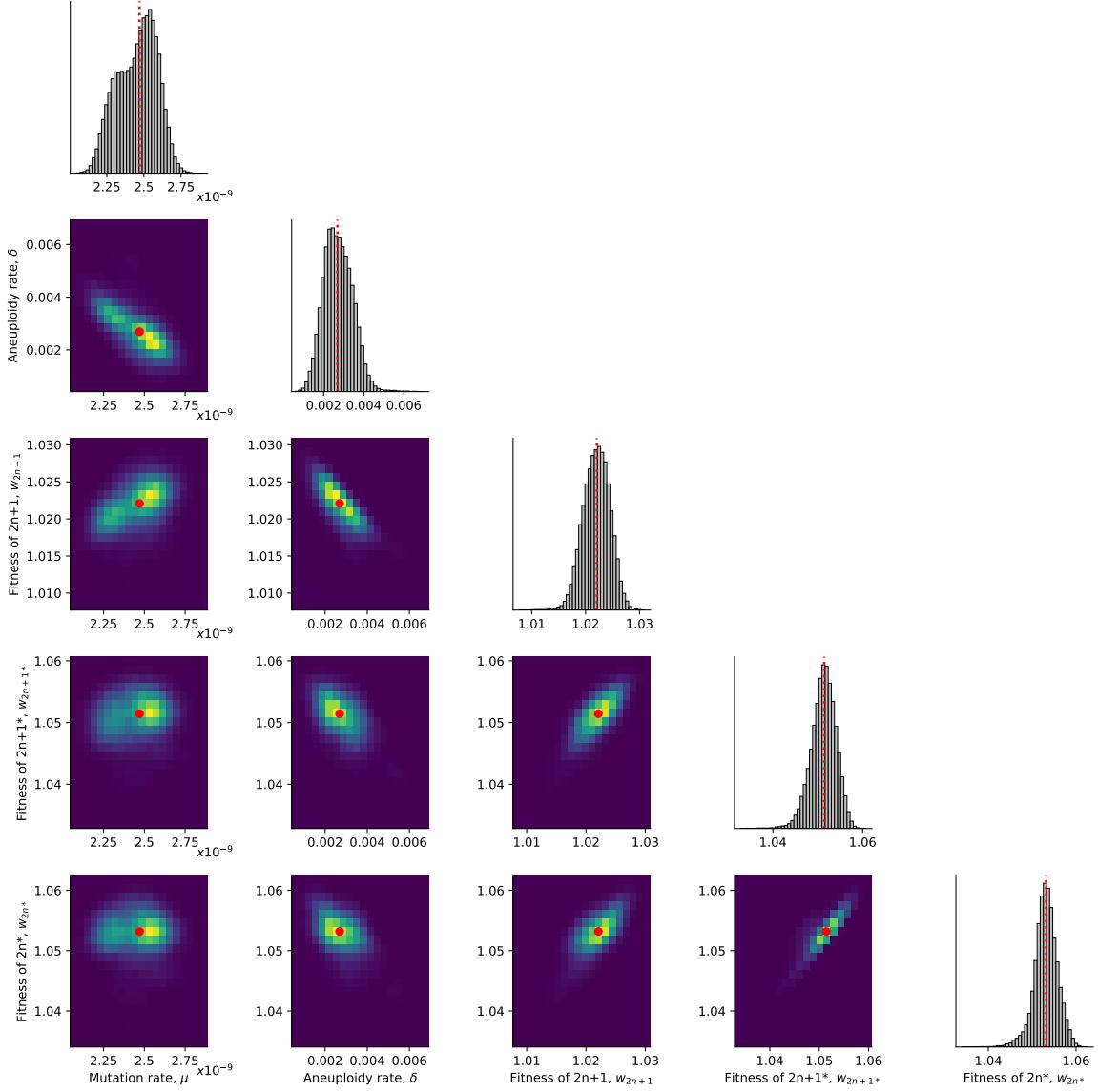
WAIC defined in eq. (6).



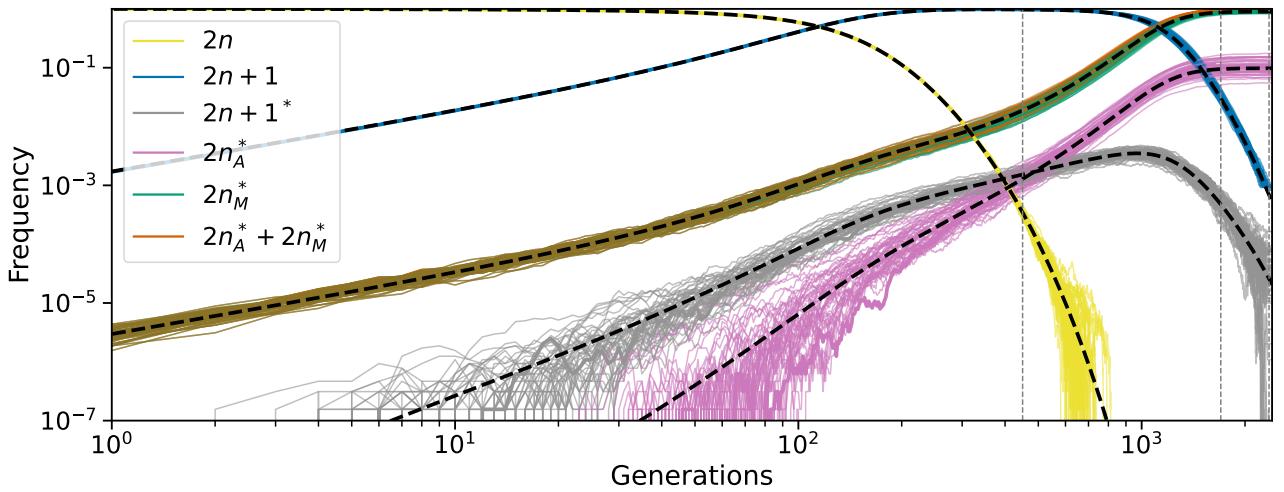
**Figure S6: Likelihood profiles.** Sensitivity of the model approximate likelihood,  $\mathcal{L}(\theta)$ , to changing a single parameter while the other parameters remain fixed at their MAP estimates. Dashed vertical line represents the MAP value. The prior distributions for the mutation rate and aneuploidy rate are  $\mu \sim U(10^{-9}, 10^{-5})$  and  $\delta \sim U(10^{-6}, 10^{-2})$ , respectively.



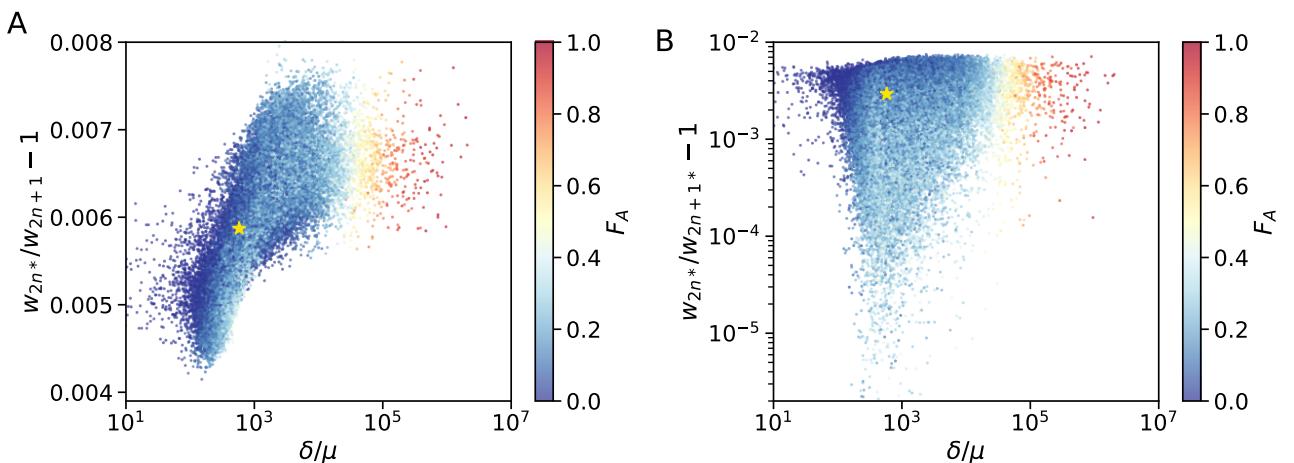
**Figure S7: Model with fixed mutation rate. (A-D)** The inferred posterior distributions for models with free and fixed mutation rate,  $\mu$ . The MAP (maximum a posteriori) and 50% HDI (highest density interval) for each model are: **free  $\mu$ , run 1:**  $\delta = 1.720 \cdot 10^{-3}$  [ $1.470 \cdot 10^{-3} - 2.786 \cdot 10^{-3}$ ],  $w_{2n+1} = 1.022$  [1.021 – 1.023],  $w_{2n+1^*} = 1.025$  [1.024 – 1.026],  $w_{2n^*} = 1.028$  [1.026 – 1.029]; **free  $\mu$ , run 2:**  $\delta = 2.129 \cdot 10^{-3}$  [ $1.334 \cdot 10^{-3} - 2.695 \cdot 10^{-3}$ ],  $w_{2n+1} = 1.022$  [1.02 – 1.023],  $w_{2n+1^*} = 1.025$  [1.023 – 1.026],  $w_{2n^*} = 1.028$  [1.026 – 1.029];  **$\mu = 10^{-5}$ :**  $\delta = 2.903 \cdot 10^{-3}$  [ $2.399 \cdot 10^{-3} - 3.156 \cdot 10^{-3}$ ],  $w_{2n+1} = 1.022$  [1.021 – 1.023],  $w_{2n+1^*} = 1.024$  [1.023 – 1.025],  $w_{2n^*} = 1.027$  [1.026 – 1.028];  **$\mu = 10^{-6}$ :**  $\delta = 1.917 \cdot 10^{-3}$  [ $9.624 \cdot 10^{-4} - 2.447 \cdot 10^{-3}$ ],  $w_{2n+1} = 1.022$  [1.02 – 1.023],  $w_{2n+1^*} = 1.025$  [1.023 – 1.026],  $w_{2n^*} = 1.028$  [1.027 – 1.029];  **$\mu = 10^{-7}$ :**  $\delta = 2.901 \cdot 10^{-3}$  [ $2.139 \cdot 10^{-3} - 3.671 \cdot 10^{-3}$ ],  $w_{2n+1} = 1.019$  [1.017 – 1.02],  $w_{2n+1^*} = 1.025$  [1.024 – 1.026],  $w_{2n^*} = 1.027$  [1.026 – 1.029].



**Figure S8: Posterior distribution of parameters inferred with the extended prior distribution.** On the diagonal, the inferred posterior distribution of each model parameter. Below the diagonal, the inferred joint posterior distribution of pairs of model parameters (dark purple and bright yellow for low and high density, respectively). Red markers and orange lines for the joint MAP estimate (which may differ from the marginal MAP, as the marginal distribution integrates over all other parameters).



**Figure S9: Posterior predicted genotype frequencies in log-log scale.** Frequency dynamics of the different genotypes with MAP parameter estimates, same as Figure 5A, but in log-log scale. Black dashed curves for a deterministic model without genetic drift. Clearly, appearance of  $2n+1$  and  $2n_M^*$  is deterministic. Appearance of  $2n+1^*$ , and therefore  $2n_A^*$ , is stochastic, however, the frequency dynamics are deterministic above a frequency of roughly 0.001. Note that the  $2n_M^*$  and the  $2n_A^* + 2n_M^*$  lines are overlapping for much of their trajectories.



**Figure S10: Posterior distribution of  $F_A$ .** (A,B)  $F_A$  values (color coded) as in Figure 5 for different parameter choices on the x- and y-axes. White star denotes the MAP estimate.