

# 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>46,36,2</sup>, and fungi<sup>39,69,43,58</sup>.  
Aneuploidy has been implicated in cancer formation, progression, and drug resistance<sup>4,48,46,45,22,32</sup>.  
38 It is also common in protozoan pathogens of the *Leishmania* genus, a major global health concern<sup>34</sup>,  
and contributes to the emergence of drug resistance<sup>49</sup> and virulence<sup>35</sup> in fungal pathogens, which  
40 are under-studied<sup>44</sup>, despite infecting a billion people per year, causing significant morbidity in >150  
million and death in >1.5 million people per year<sup>49,44</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>52</sup>. For example, aneuploid mouse embryonic cells grow slower than euploid cells<sup>63</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>37,61,39,52,47,26,68,62,64</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>39,64</sup>. Indeed, in a survey of 1,011 yeast strains,  
50 aneuploidy has been detected in about 19%<sup>40</sup>. Thus, aneuploidy can lead to rapid adaptation in  
unicellular eukaryotes<sup>16,60,20,42</sup>, as well as to rapid growth of somatic tumour cells<sup>48,54</sup>. For example,  
52 aneuploidy in *Saccharomyces cerevisiae* facilitates adaptation to a variety of stressful conditions  
like heat and pH<sup>66</sup>, copper<sup>8,16</sup>, salt<sup>11</sup>, and nutrient limitation<sup>12,18,1</sup>, with similar results in *Candida*  
54 *albicans*<sup>64</sup>. Importantly, aneuploidy can also lead to drug resistance in pathogenic fungi such as  
*C. albicans*<sup>51,50,15</sup> and *Cryptococcus neoformans*<sup>55</sup>, which cause candidiasis and meningoencephalitis,  
56 respectively.

Yona et al.<sup>66</sup> demonstrated experimentally the importance of aneuploidy in adaptive evolution. They  
58 evolved populations of *S. cerevisiae* under strong heat stress. The populations adapted to the heat stress  
within 450 generations, and this adaptation was determined to be due a duplication of Chromosome  
60 III. Later on, after more than 1,500 generations, the populations reverted back to an euploid state, while  
remaining adapted to the heat stress. Aneuploidy was therefore suggested to be a *transient adaptive*  
62 *solution*, because it can rapidly appear and fixate in the population under stressful conditions, and can  
then be rapidly lost when the cost of aneuploidy outweighs its benefit—after the stress is removed,  
64 or after "refined" beneficial mutations appear and fixate<sup>66</sup>. Furthermore, it has been suggested that  
aneuploidy is an evolutionary "stepping stone" that facilitates future adaptation by genetic mutations,

66 which require more time to evolve<sup>66,65</sup>.

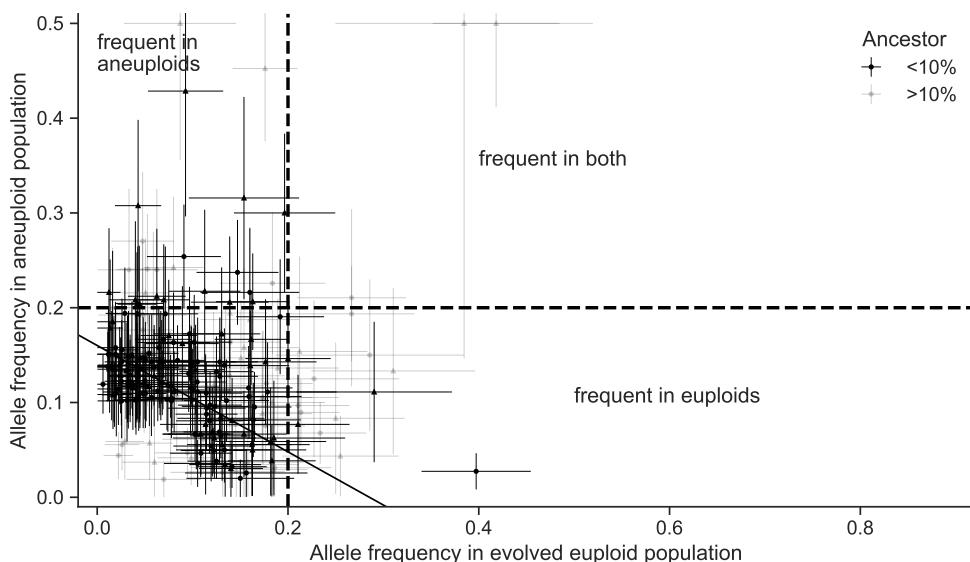
Here, we test the hypothesis that aneuploidy is a *an evolutionary stepping stone* that facilitates adaptive evolution by genetic mutations Yona et al.<sup>65</sup>. First, we sequenced the genomes of evolved populations reported in<sup>66</sup> and analyzed their mutant allele frequencies to assess if the evolved euploid cells are descended from aneuploid cells. Second, we develop an evolutionary genetic model and fit it to the experimental results of Yona et al.<sup>66</sup> in order to predict the genotype frequency dynamics in the experimental populations, thereby estimating the frequency of evolved euploid cells that descended 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 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 the path to adaptation.

## 78 Results

In the heat-stress experiment of Yona et al.<sup>66</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, 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 generation 1,700 and 2,350 in *H4* and *H2*, respectively.

84 **Empirical frequencies of mutant alleles.** For each of two evolved populations (*H2* and *H4*) we 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 (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 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*. 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 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 *decreasing* heat tolerance<sup>24</sup>.

96 Surprisingly, out of all these mutant alleles, none was present at a frequency >20% in both the  
 97 aneuploid and the evolved euploid populations. More importantly, a high mutant allele frequency  
 98 in the aneuploid population was associated with a low frequency in the evolved euploid population,  
 99 and vice-versa (Spearman's correlation coefficient  $\rho = -0.64$  and  $-0.66$  in the two experimental  
 100 repetitions; Figure 1), suggesting that mutant alleles frequent in the aneuploid populations decreased  
 101 in frequency when aneuploidy was lost. These results suggest evolved euploid cells are unlikely to  
 102 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>66</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%).

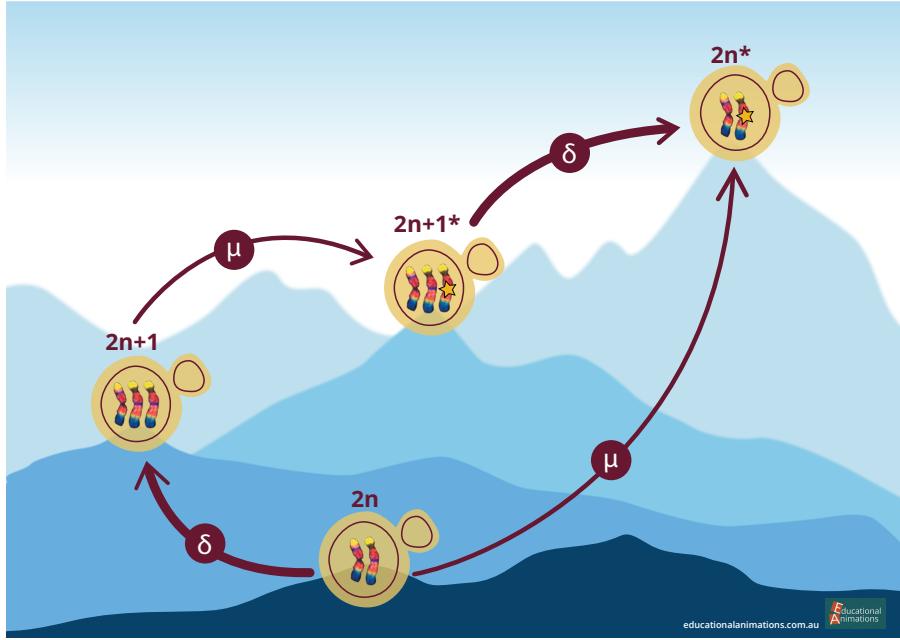
**Evolutionary genetic model.** To explore the dynamics during the evolutionary experiments, we  
104 developed an evolutionary genetic model, fitted the model to empirical data, and used it to predict the  
genotype frequency dynamics, or specifically, the fraction of the evolved euploid population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

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

114 We fitted this model to the experimental results<sup>66</sup> – time for fixation (>95%) and for loss (<5%) of  
aneuploidy – using approximate Bayesian computation with sequential Monte Carlo (ABC-SMC)<sup>56</sup>,  
116 thereby inferring the model parameters: rates aneuploidy and mutation and the fitness of all genotypes.  
We then sampled posterior predictions for the genotype frequency dynamics using the estimated  
118 parameter values and compared different versions of the model to test additional hypotheses about the  
evolutionary process.

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

124 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  
generation. From the literature, the mutation rate per base pair is roughly  $2 - 3 \cdot 10^{-10}$  (refs. <sup>70,33</sup>), but  
126 it may be higher under heat stress, as several stresses<sup>19</sup>, including heat<sup>21</sup>, may cause hypermutation  
in yeast. If we assume a 10-fold increase over the mutation rate reported in the literature, then the  
128 estimated beneficial mutation rate can be explained by a genomic target size of 1,000 base pairs  
(that is, 1,000 base pairs across the genome in which a mutation would provide a fitness benefit).  
130 Supporting this, Jarolim et al.<sup>24</sup> found 279 genes that contributed to survival after a sudden shift from  
30 °C to 50 °C, and Flynn et al.<sup>13</sup> used a deep mutational scan of a single protein, Hsp90, to find 465  
132 amino-acid variants that increased growth rate in 37 °C. Furthermore, Yona et al.<sup>66</sup> found at least 10  
genes on Chromosome III that increased heat tolerance when over-expressed. Assuming that other



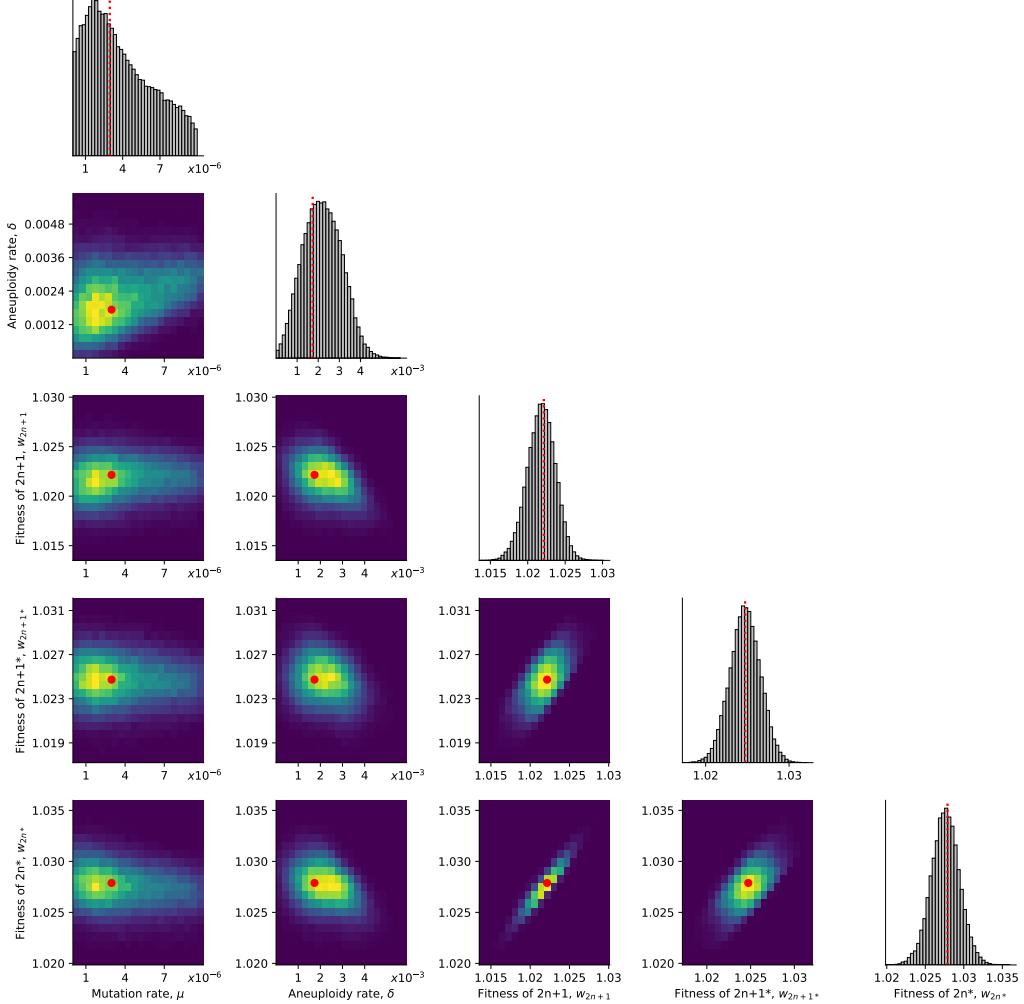
**Figure 2: Model Illustration.** There are four genotypes in our model: euploid wild-type,  $2n$ ; euploid mutant,  $2n+1$ ; aneuploid wild-type,  $2n+1^*$ ; and aneuploid mutant,  $2n^*$ . Overall there are two possible trajectories from  $2n$  to  $2n^*$ . Arrows denote transitions between genotypes, with transitions 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.

134 chromosomes also have a similar number of heat-tolerance genes (and even more, as Chromosome  
 III is one of the smallest chromosomes<sup>17</sup>), we get a total of 160 heat-tolerance genes in the genome.  
 136 Indeed, mutations were found in 97 genes in an evolutionary experiment with yeast under heat stress<sup>21</sup>.  
 Thus, to get a genomic target size of 1,000, it is enough that the average gene target size (number of  
 138 base pairs in a gene in which a mutation is beneficial) is 6.25 base pairs. For example, Kohn and  
 Anderson<sup>29</sup> found a target size of 11 in a proton exporter gene (*PMA1*) that contributes to high-salt  
 140 adaptation.

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  
 142 studies: for Chromosome III in diploid *S. cerevisiae*, Zhu et al.<sup>70</sup> estimated  $6.7 \cdot 10^{-6}$  chromosome  
 gain events per generation, and Kumaran et al.<sup>31</sup> estimate  $3.0 \cdot 10^{-5} - 4.3 \cdot 10^{-5}$  chromosome loss  
 144 events per generation (95% confidence interval). However, this difference may be partly explained  
 by an increased aneuploidy rate during heat stress: heat shock can increase the rate of chromosome  
 146 fragment loss by 2-3 orders of magnitude<sup>5</sup>.

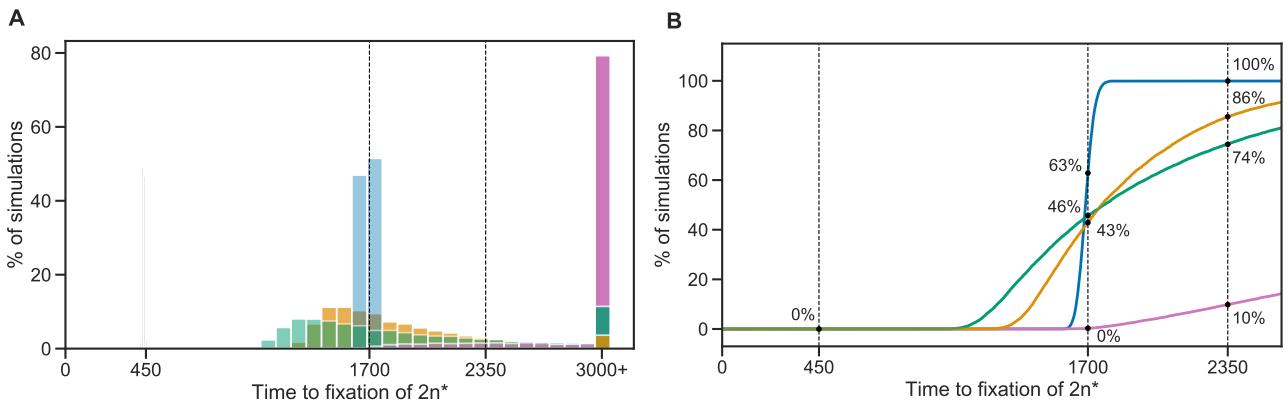
The estimated fitness values are  $w_{2n+1} = 1.022$  [ $1.021 - 1.023$ ],  $w_{2n+1^*} = 1.025$  [ $1.024 - 1.026$ ],  
 148  $w_{2n^*} = 1.028$  [ $1.026 - 1.029$ ], all relative to the fitness of  $2n$ , which is set to  $w_{2n} = 1$ . Thus, we  
 can infer that the cost of Chromosome III trisomy is  $c = w_{2n^*} - w_{2n+1^*} = 0.003$  (or 0.3%) and the

- 150 benefit of trisomy is  $w_{2n+1} - 1 - c = 0.019$  (1.9%), whereas the benefit of the beneficial mutation is  $w_{2n^*} - 1 = 0.028$  (2.8%).
- 152 If we allow for transitions (mutation, chromosome loss and gain) to less-fit genotypes (e.g.,  $2n^*$  to  $2n+1^*$ ), 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).

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



**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>66</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.

- 158 However, a model without aneuploidy (where the aneuploidy rate is fixed at zero,  $\delta = 0$ ), fails to  
 explain the experimental observations (Figure 4). The estimated mutation rate without aneuploidy is  
 160  $\mu = 7.98 \cdot 10^{-9}$  [ $7.906 \cdot 10^{-9} - 8.138 \cdot 10^{-9}$ ], much lower compared to a model with aneuploidy. The  
 fitness of the mutant is also much lower at  $w_{2n}^* = 1.013$  [1.012 – 1.013]. This is because, without  
 162 aneuploidy, a high mutation rate or fitness effect will lead to faster appearance and fixation of  $2n^*$  than  
 in the experimental observations.
- 164 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  
 166 with no aneuploidy (in which  $\delta = 0$ ), but worse than a model with positive selection for aneuploidy,  
 in which  $w_{2n} < w_{2n+1} < w_{2n+1}^* < w_{2n}^*$  (Figure 4).

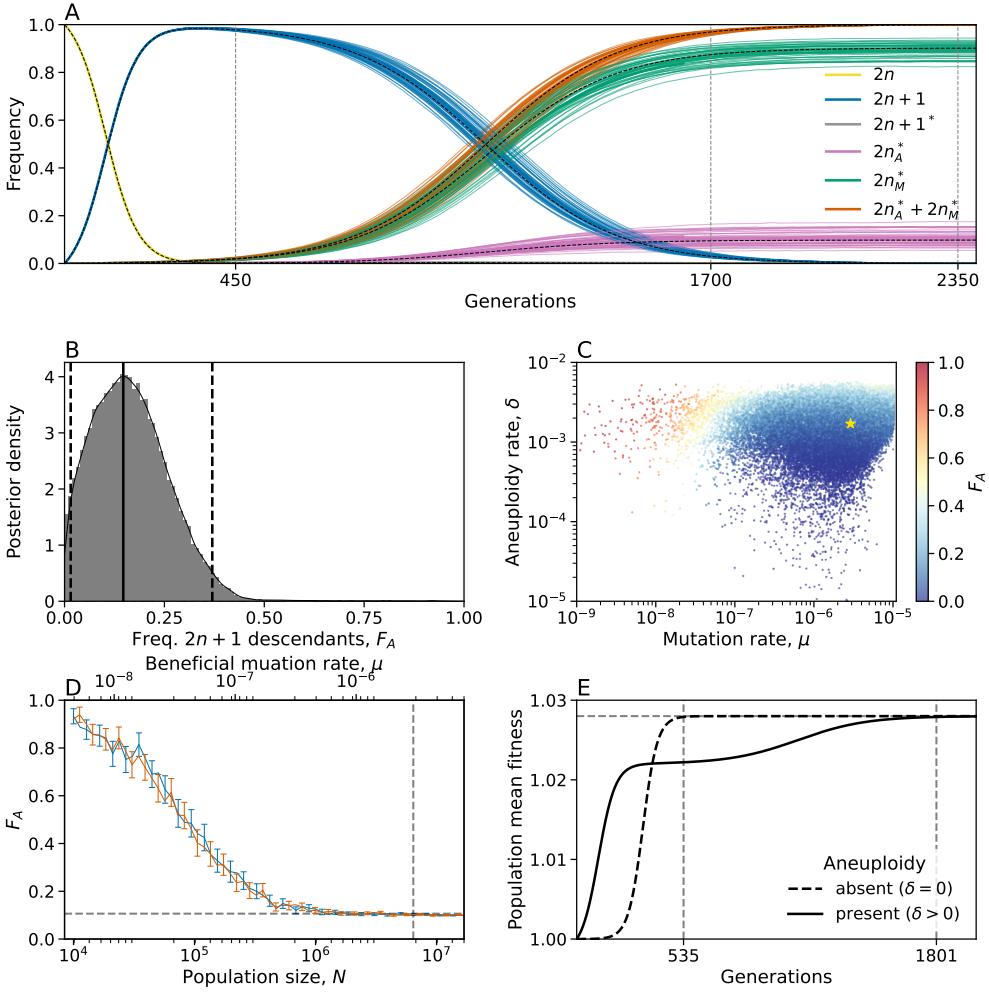
168 **Model predictions of genotype frequency dynamics.** We simulated 50 replicate genotype frequency dynamics using the MAP estimate parameters. Figure 5A shows the simulated frequencies of  
170 the four genotypes ( $2n$ ,  $2n+1$ ,  $2n+1^*$  and  $2n^*$ ), as well as the frequencies of  $2n^*$  cells that arose from either  $2n+1$  cells via a sequences of mutation and chromosome loss events ( $2n_A^*$ ), or directly from  
172  $2n$  cells via a mutation event ( $2n_M^*$ ). We find that  $2n+1^*$  never reaches substantial frequency as it is quickly replaced by  $2n^*$  in a process similar to *stochastic tunneling*<sup>23,30</sup>.

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

180 This result is not unique to the MAP parameter estimate. We simulated genotype frequency dynamics using parameter samples from the posterior distribution, and computed the posterior distribution of  $F_A$   
182 (Figure 5B). The posterior mode  $F_A$  was just 0.147 [0.0154-0.370 95% CI] and only in 489 of 100,000 posterior samples (0.489%)  $F_A$  was larger than 0.5 (see Supporting Material for results when transitions  
184 to less-fit genotypes are allowed, such as  $2n^*$  to  $2n+1^*$ ). Thus, if we sample a random cell from the evolved  $2n^*$  population, it is more likely to have descended directly from an euploid cell than from  
186 an aneuploid cell. The probability of  $2n^*$  descending from  $2n+1$  ( $F_A$ ) increases with the aneuploidy rate,  $\delta$ , and decreases with both the population size  $N$  and the mutation rate,  $\mu$  (Figure 5C,D). In some  
188 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>53</sup> have demonstrated a fold increase of between 2.2 and 7.1 in mutation rate. Therefore, we inferred model parameters under the assumption that the mutation rate increases  
192 in aneuploid cells by a factor  $\tau = 1, 33/32$  (due to an additional chromosome), 2, 5, 10, or 100 (due to genetic instability). We found that the posterior distribution was similar for  $\tau = 1, 33/32, 2$ , and 5  
194 (Figure S4). Furthermore, we computed the WAIC, a criterion for model selection (Methods). The WAIC values were similar for all  $\tau$  values (Table S3).

196 Assuming a strong increase of the mutation rate in aneuploid cells, i.e.  $\tau = 100$ , the inferred a mutation rate was  $\mu = 4.094 \cdot 10^{-7}$  [ $6.252 \cdot 10^{-8} - 6.046 \cdot 10^{-7}$ ]), and the inferred aneuploidy rate  
198 that was  $\delta = 0.744 \cdot 10^{-3}$  [ $0.506 \cdot 10^{-3} - 1.827 \cdot 10^{-3}$ ]. Compared to inference made assuming no



**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.

effect of aneuploidy on the mutation rate, these rates were about 7-8-fold and 2-3-fold lower for  $\mu$  and  
200  $\delta$ , respectively. Assuming  $\tau = 10$ , the inferred a mutation rate was only slightly lower compared to  
 $\tau = 1 (\mu = 1.67 \cdot 10^{-6} [2.836 \cdot 10^{-8} - 2.245 \cdot 10^{-6}])$ .

202 Therefore, we do not find any evidence of an increase in mutation rate in aneuploid cells. This may  
be because, unless the increase is strong ( $\tau \geq 10$ ), it does not seem to affect our inference; or because  
204 Chromosome III is one of the smallest chromosomes<sup>17</sup>. We also checked the differences in genotype  
frequency dynamics for different  $\tau$  values. We observe  $\tau = 100$  could be distinguished if accurate  
206 data was available for the waiting time until the frequency of  $2n$  to decrease below 95% (Figure S5A)  
or for waiting time for the frequency of  $2n+1$  to either reach or go below 95% (Figure S5B). Similarly,  
208 we did not find evidence for an increase in the aneuploidy rate in aneuploid cells<sup>53</sup>, probably due to  
lack of statistical power.

## 210 Discussion

In a study on the role of chromosome duplication in adaptive evolution, Yona et al.<sup>66</sup> found that a  
212 Chromosome III trisomy was acquired by *S. cerevisiae* populations evolving under heat stress, only  
to be later replaced by euploid mutant cells that carry "refined" solutions to the stress. Additionally,  
214 such a replacement also occurred when they initiated evolutionary experiments with a population in  
which all cells carry a Chromosome III trisomy. They hypothesized that aneuploidy is a "useful yet  
216 short-lived intermediate that facilitates further adaptation", suggesting that the euploid mutant cells  
evolved by heat-resistance mutations in aneuploid cells followed by reversion of trisomy due to a  
218 chromosome loss event.

If indeed the evolved euploid population is descended from the aneuploid population, then mutant  
220 alleles that were common in the aneuploid populations should also be common in the evolved euploid  
population. However, this is likely not the case (Figure 1): mutant allele frequencies in the aneuploid  
222 and euploid populations are negatively correlated, such that common alleles in the former are rare  
in the latter. Furthermore, we developed an evolutionary genetic model of adaptive evolution by  
224 aneuploidy and mutation (Figure 2), fitted it to the experimental results of Yona et al.<sup>66</sup>, and used  
it to predict the genotype frequency dynamics. The model predicted that only about 10-15% of the  
226 evolved euploid population descended from aneuploid cells by acquiring a mutation and losing the  
extra chromosome—that is, the majority of the euploid population are not descended from aneuploid  
228 cells, but rather are direct descendants of the ancestral wild-type population (Figure 5).

This happens despite aneuploidy reaching a high frequency in the population (>95%). Conventional wisdom might suggest that once the aneuploid genotype  $2n+1$  reaches high frequency, it will have a better chance at producing "refined" solutions via mutations, and its descendants will come to dominate the population: the frequency of  $2n_A^*$  (which arises from  $2n+1^*$ ) will be higher than the frequency of  $2n_M^*$  (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<sup>69</sup>). 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>3,6,67,22</sup>. However, in the experiment of Yona et al.<sup>66</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>65</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

288 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 °C (ancestral diploid) or 39 °C (evolved  
290 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.  
292 Genomic DNA was extracted using "MasterPure Yeast DNA Purification Kit" (Lucigen) according to  
the manufacture instructions. Following extraction, DNA concentrations were determined by Qubit  
294 assay (Thermo Fisher) and ~ 1 $\mu$ g DNA was used for library preparation using Illumina sample  
preparation kit (Illumina). Samples were sequenced using a 100 bp pair end read output run using  
296 Illumina HiSeq2500.

**Evolutionary genetic model.** We model the evolution of a population of cells using a Wright-Fisher model<sup>38</sup>, assuming a constant effective population size  $N$ , non-overlapping generations, and including the effects of natural selection, genetic drift, aneuploidy, and mutation. We focus on beneficial genetic modifications, neglecting the effects of deleterious and neutral mutations or karyotypic 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): 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 chromosome duplication; and aneuploid mutant,  $2n+1^*$ , with an extra chromosome and a beneficial mutation.

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., 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 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 aneuploidy rates in aneuploid cells (as in Sheltzer et al.<sup>53</sup>) did not perform well, probably due to lack of statistical power, and was abandoned.

In the experiment by Yona et al.<sup>66</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  $N = 6.425 \cdot 10^6$ , 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

by

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

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

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

Finally, random genetic drift is modeled using a multinomial distribution<sup>38</sup>,

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

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>66</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,

$$\begin{aligned} \mathcal{L}(\theta) &= P^4(200 \leq T(2n+1) \leq 450) \cdot \left[ 1 - \right. \\ 344 \quad &\quad P_{\tilde{\mathbf{X}}}^4(\{T(2n^*) < 1700\} \mid 200 \leq T(2n+1) \leq 450) - \\ &\quad P_{\tilde{\mathbf{X}}}^4(\{1700 < T(2n^*) < 2350\} \mid 200 \leq T(2n+1) \leq 450) + \\ &\quad \left. P_{\tilde{\mathbf{X}}}^4(\{T(2n^*) < 1700\} \wedge \{1700 < T(2n^*) < 2350\} \mid 200 \leq T(2n+1) \leq 450) \right], \end{aligned} \quad (4)$$

where  $!\{\dots\}$  is the "logical not" operator,  $P^4(\dots)$  is the 4th power of  $P(\dots)$ , and all probabilities

346  $P_{\tilde{\mathbf{X}}}(\dots)$  are approximated from the results of the simulations  $\tilde{\mathbf{X}}$ . For example,  $P_{\tilde{\mathbf{X}}}(\{T(2n^*) < 1700\} \mid$   
 348  $200 \leq T(2n+1) \leq 450)$  is approximated by taking simulations in which  $2n+1$  fixed before generation  
 350 450 but not before generation 200, and computing the fraction of such simulations in which  $2n^*$  did  
 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 likely sufficient.

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

$$\begin{aligned} 356 \quad \mathcal{L}_!(\theta) &= 1 - P_{\tilde{\mathbf{X}}}^4(\{T(2n^*) < 1700\}) - \\ &\quad P_{\tilde{\mathbf{X}}}^4(\{1700 < T(2n^*) < 2350\}) + \\ &\quad P_{\tilde{\mathbf{X}}}^4(\{T(2n^*) < 1700\} \wedge \{1700 < T(2n^*) < 2350\}). \end{aligned} \quad (5)$$

**Parameter inference.** To infer model parameters, we use approximate Bayesian computation with

358 a sequential Monte-Carlo scheme, or ABC-SMC<sup>56</sup>, implemented in the pyABC Python package<sup>28</sup>  
[pyabc.readthedocs.io](http://pyabc.readthedocs.io). This approach uses numerical stochastic simulations of the model to infer  
 360 a posterior distribution over the model parameters. It is a method of likelihood-free, simulation-  
 based inference<sup>9</sup>, that is, for estimating a posterior distribution when a likelihood function cannot be  
 362 directly computed. It is therefore suitable in our case, in which the likelihood function can only be  
 approximated from simulations, and cannot be directly computed.

364 The ABC-SMC algorithm employs sequential importance sampling over multiple iterations<sup>59,27,57</sup>. In  
 iteration  $t$  of the algorithm, a set of parameter vectors,  $\{\theta_{i,t}\}_{i=1}^{n_t}$ , also called *particles*, are constructed  
 366 in the following way. A proposal particle,  $\theta^*$ , is sampled from a proposal distribution, and is either  
 accepted or rejected, until  $n_t$  particles are accepted. The number of particles,  $n_t$ , is adapted at every

368 iteration  $t$  using the adaptive population strategy<sup>28</sup> [pyabc.readthedocs.io](https://pyabc.readthedocs.io). For  $t = 0$ , the proposal  
369 particle is sampled from the prior distribution,  $p(\theta)$ . For  $t > 0$ , the proposal particle is sampled from  
370 the particles accepted in the previous iteration,  $\{\theta_{i,t-1}\}_{i=1}^{n_{t-1}}$ , each with a probability relative to its weight  
371  $W_{t-1}(\theta_{i,t-1})$  (see below). The proposal particle is then perturbed using a kernel perturbation kernel,  
372  $K_t(\theta^* | \theta)$  where  $\theta$  is the sample from the previous iteration. Then, a set of synthetic observations  
373  $\tilde{\mathbf{X}}^*$  is simulated, and the proposal particle  $\theta^*$  is accepted if its approximate likelihood (eq. (4)) is high  
374 enough,  $\mathcal{L}(\theta^*) > 1 - \epsilon_t$  (or more commonly, if  $1 - \mathcal{L}(\theta^*) < \epsilon_t$ ), where  $\epsilon_t > 0$  is the *acceptance*  
375 *threshold*, as higher values of  $\epsilon_t$  allow more particles to be accepted. The acceptance threshold  $\epsilon_t$   
376 is chosen as the median of the  $1 - \mathcal{L}(\theta)$  of the particles accepted in the previous iteration,  $t - 1$ ,  
377 and  $\epsilon_0 = 0.01$ . For each accepted particle  $\theta_{i,t}$  a weight  $W_t(\theta_{i,t})$  is assigned: for  $t = 0$ ,  $W_0(\theta_{i,0}) = 1$ ,  
378 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  $\theta$   
379 and  $K_t(\theta', \theta)$  is the probability of a perturbation from  $\theta$  to  $\theta'$ .  $K_t(\theta' | \theta)$  is a multivariate normal  
380 distribution, fitted at iteration  $t$  to the particles from the previous iteration,  $\{\theta_{i,t-1}\}_{i=1}^{n_{t-1}}$ , and their  
381 weights,  $\{W(\theta_{i,t-1})\}_{i=1}^{n_{t-1}}$ .

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

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 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 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 distribution with histograms.

396 **Model comparison.** We examine several versions of our evolutionary models, e.g. without aneuploidy or with increased mutation rate in aneuploid cells, as well as several different prior distributions  
397 (see below). To compare these, we plot posterior predictions: for each model we execute 10,000  
398 simulations using the MAP parameter estimates and plot the distributions of time to fixation of  $2n^*$ ,

400 one of key properties of the model likelihood. These plots visualize the fit of each model to the  
401 data. Also, for similar models we plot the marginal and joint posterior distributions of the parameters;  
402 if these are similar, we consider the models interchangeable. We validate this by comparing HDI  
(highest density interval) of posterior distributions.

404 Where posterior plots are very similar and the number of parameters is the same, we use WAIC, or  
the widely applicable information criterion<sup>14</sup>, defined as

406 
$$WAIC(\theta) = -2 \log \mathbb{E}[\mathcal{L}(\theta)] + 2\mathbb{V}[\log \mathcal{L}(\theta)] \quad (6)$$

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  
KDE. We validated that upon resampling WAIC values do not significantly change and that differences  
410 in WAIC between models are preserved. WAIC values are scaled as a deviance measure: lower values  
imply higher predictive accuracy.

412 **Prior distributions.** We used informative prior distributions for  $w_{2n+1} = 1 - c + b$ ,  $w_{2n+1*} =$   
 $(1+s)(1-c)+b$  and  $w_{2n*} = 1+s$ , which we estimated from growth curves data from mono-culture growth  
414 experiments previously reported by Yona et al.<sup>66</sup>, Figs. 3C, 4A, and S2. We used Curveball, a method  
for predicting results of competition experiments from growth curve data<sup>41</sup> [curveball.yoavram.com](http://curveball.yoavram.com).

416 Briefly, Curveball takes growth curves of two strains growing separately in mono-culture and predicts  
how they would grow in a mixed culture, that is, it predicts the results of a competition assay. From these  
418 predictions, relative fitness values can be computed. Because Curveball uses a maximum-likelihood  
approach to estimate model parameters, we were able to estimate a distribution of relative fitness  
420 values to be used as a prior distribution by sampling 10,000 samples from a truncated multivariate  
normal distribution defined by the maximum-likelihood covariance matrix (Figure S3).

422 We used growth curves of  $2n$  and  $2n+1$  in 39 °C to estimate an informative prior distribution for  
 $w_{2n+1}$  (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  $w_{2n*} > w_{2n+1*} > w_{2n+1} > w_{2n}$ ;  
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;  
this prior distribution proved to be less useful.

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

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

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# Supplementary Material

## 620 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).

**628 Extended informative prior distribution.** In an extended informative prior distribution, we used additional growth curves of  $2n^*$  (*refined* strain from Yona et al.<sup>66</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  $2n^*$ .

**634** 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, 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 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:  $\delta = 2.705 \cdot 10^{-3}$  [ $2.094 \cdot 10^{-3} - 3.094 \cdot 10^{-3}$ ],  $w_{2n+1} = 1.022$  [ $1.021 - 1.024$ ],  $w_{2n+1^*} = 1.052$  [ $1.05 - 1.054$ ],  $w_{2n^*} = 1.053$  [ $1.051 - 1.055$ ], the latter two being much higher compare to the main informative prior. Notably, the mode of the posterior ratio  $w_{2n^*}/w_{2n+1} = 1.0009$  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 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

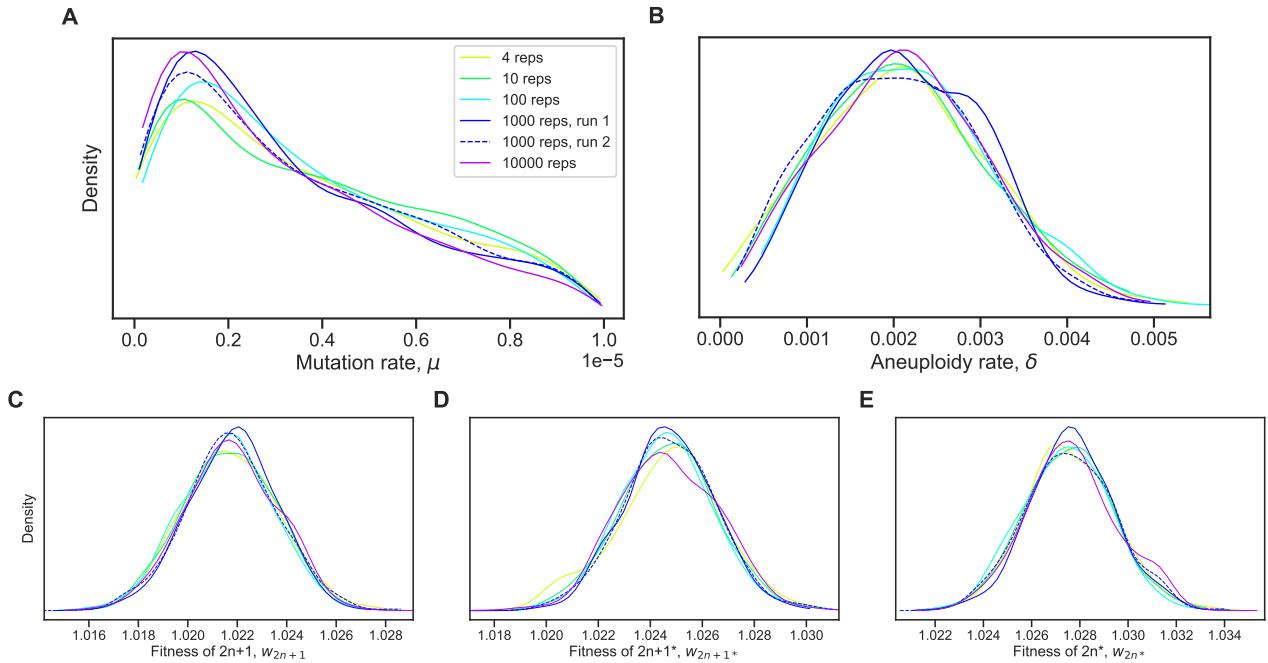
648 model that includes transitions (mutation, chromosome loss and gain) to less-fit genotypes (e.g.,  $2n^*$  to  $2n+1^*$ ),

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

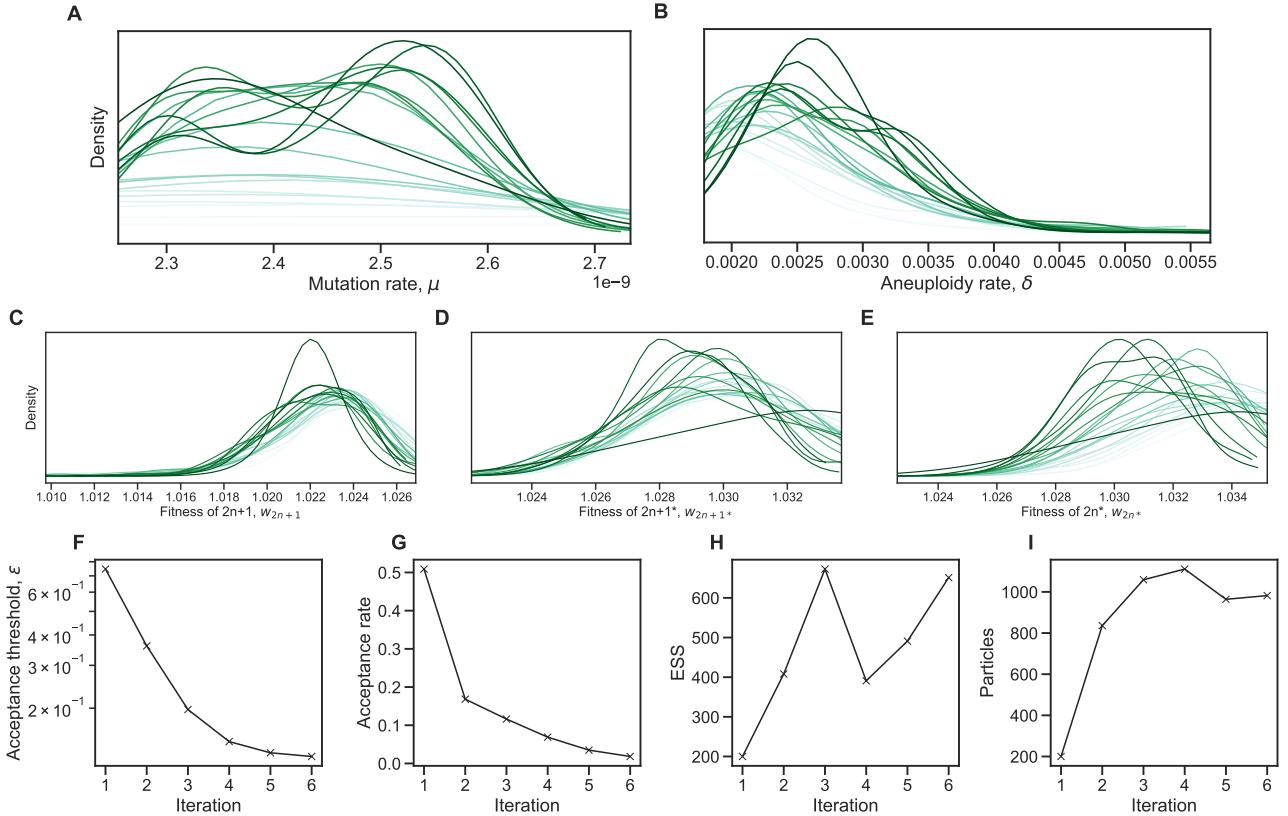
The inferred values are slightly different. The estimated mutation rate,  $\mu = 1.036 \cdot 10^{-7}$  [ $8.01 \cdot 10^{-8} - 1.339 \cdot 10^{-7}$ ], corresponds to a mutation target size of  $\sim 300 - 500$ , assuming the mutation rate per base pair is roughly  $2 \cdot 10^{-10}$  (ref.<sup>70</sup>) or  $3.3 \cdot 10^{-10}$  (ref.<sup>33</sup>). The estimated aneuploidy 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: for Chromosome III in diploid *S. cerevisiae*, Zhu et al.<sup>70</sup> estimated  $6.7 \cdot 10^{-6}$  chromosome gain events per generation, and Kumaran et al.<sup>31</sup> estimate  $3.0 - 4.3 \cdot 10^{-5}$  chromosome loss events per generation (95% confidence interval). The estimated fitness values are  $w_{2n+1} = 1.024$  [ $1.023 - 1.025$ ],  $w_{2n+1}^* = 1.025$  [ $1.024 - 1.026$ ],  $w_{2n} = 1.032$  [ $1.031 - 1.033$ ], all relative to the fitness of  $2n$ , which 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%) 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%).

662 We simulated genotype frequency dynamics using parameter samples from the posterior distribution, and computed the posterior distribution of  $F_A$ . The mean  $F_A$  in this case is just 0.0189 [0.0004 - 0.1214  
664 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 samples,  
666 none had  $F_A$  above 0.05 (i.e., 5% of the population).

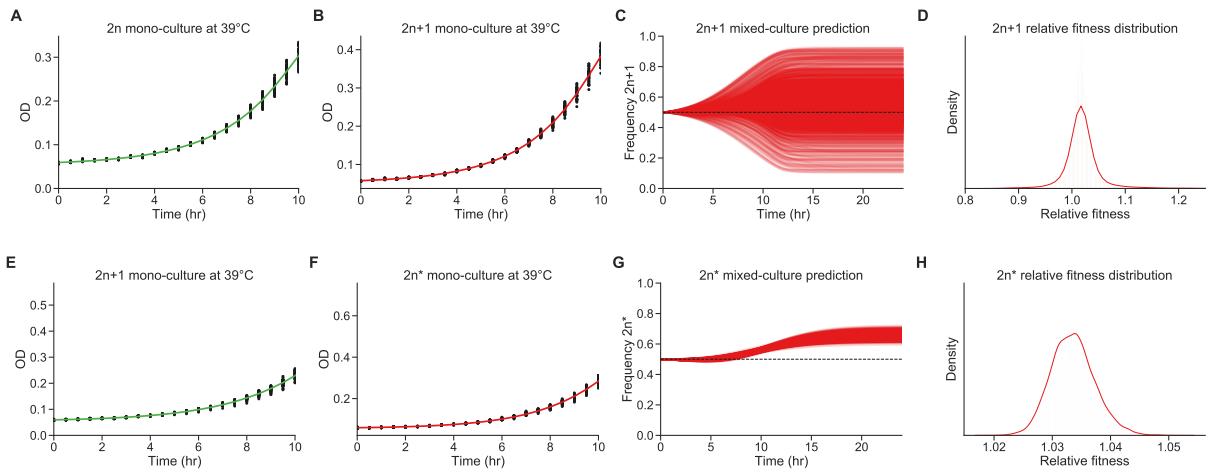
## Supplementary Figures & Tables



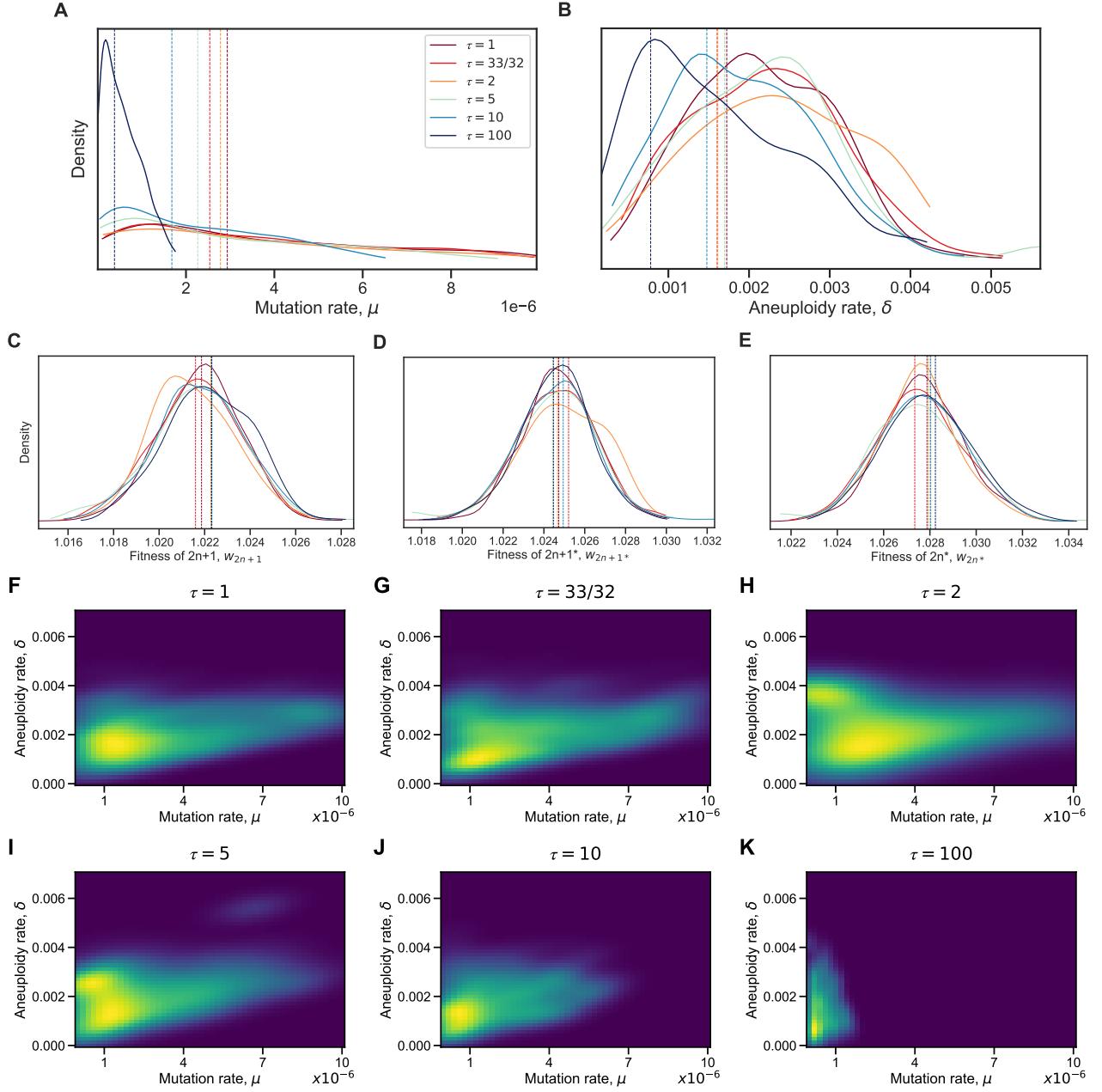
**Figure S1: Posterior distribution validation.** The posterior distribution of model parameters is roughly the same regardless of the number of simulations (4-10,000 replicates) used to approximate the likelihood (eq. (4)).



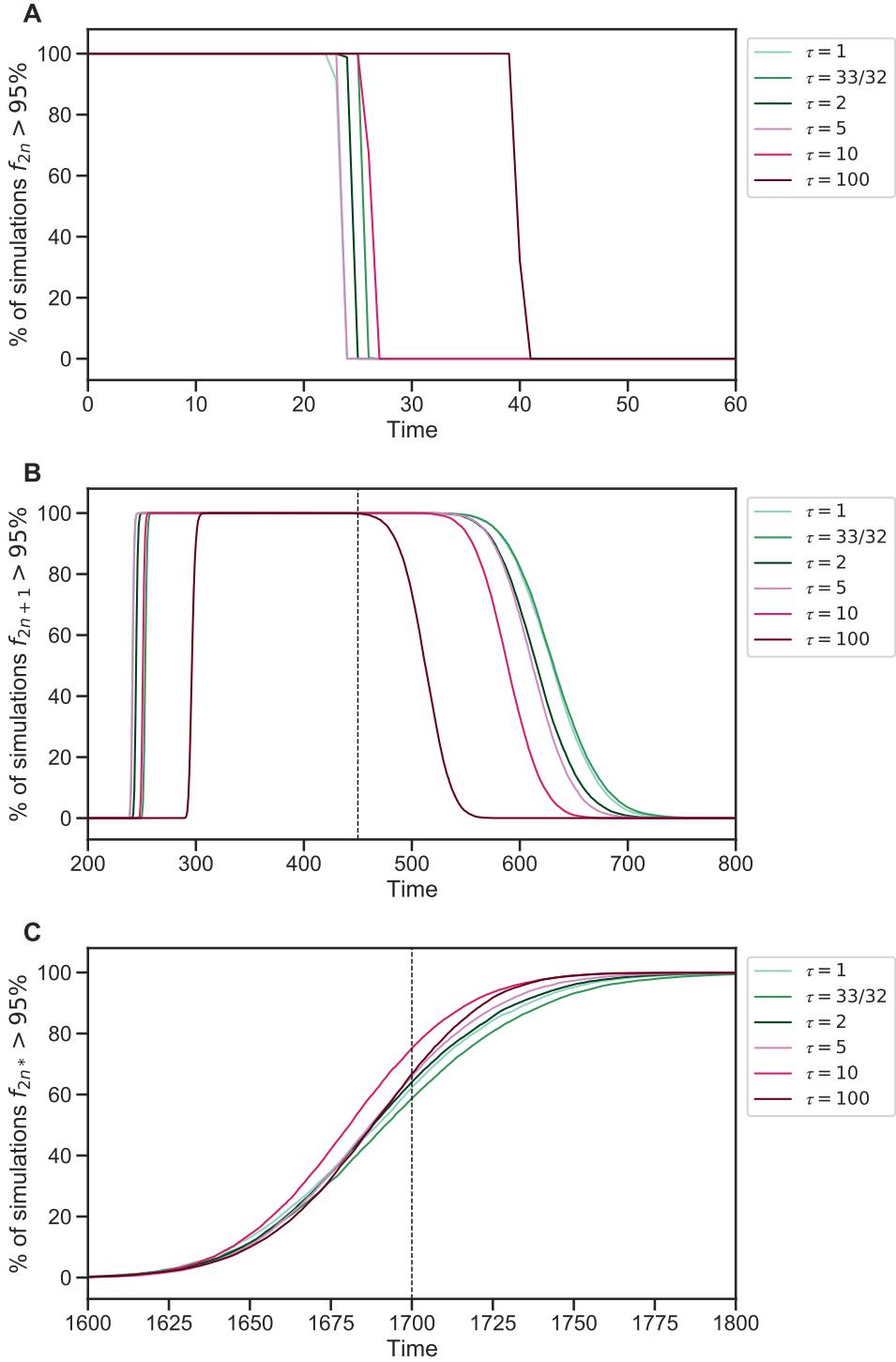
**Figure S2: Inference convergence.** The ABC-SMC algorithm was used to infer the model parameters. **(A-E)** The approximate posterior distributions of model parameters at each iteration of the ABC-SMC algorithm demonstrates convergence, as the posterior did not significantly change after the first 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>66</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>41</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.

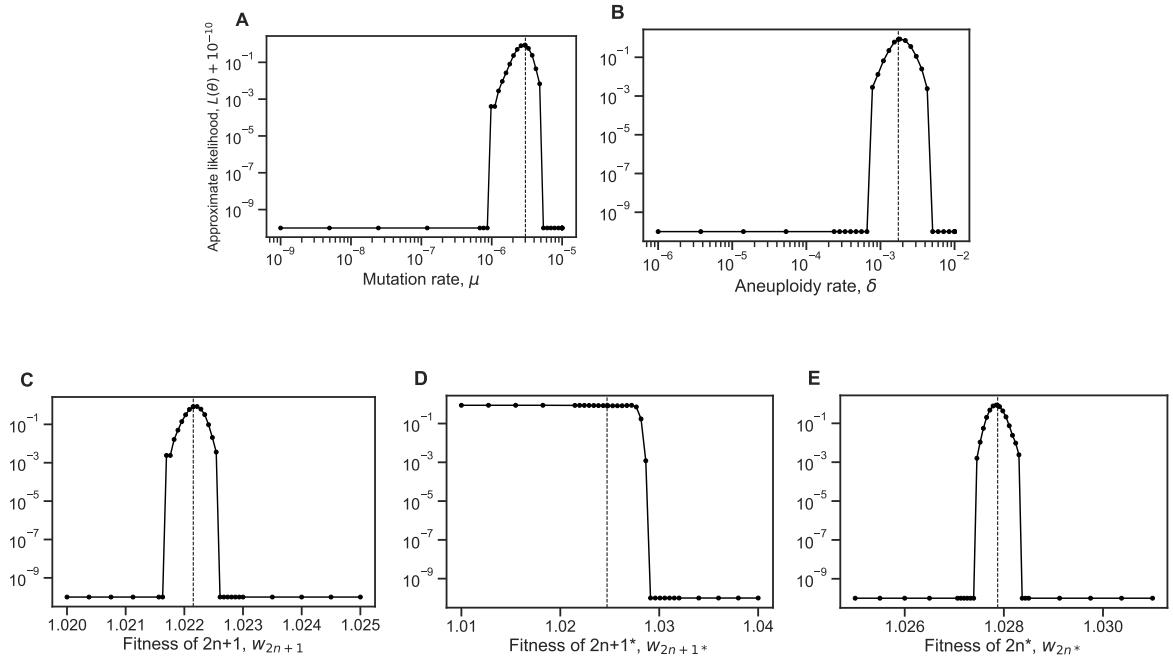
**Table S2: Mutant alleles in population  $H4$ .**

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

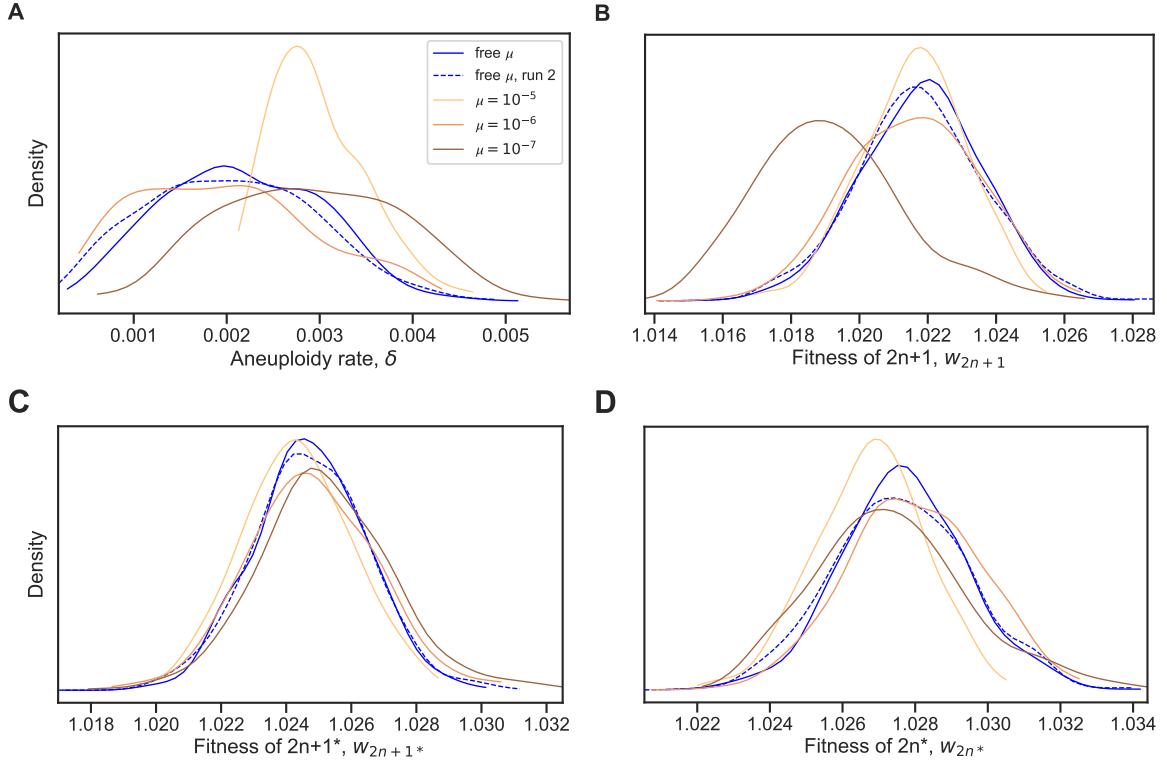
**Table S3: WAIC values for different  $\tau$  values.** Differences of less than 6 are considered of weak significance<sup>25</sup>.

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

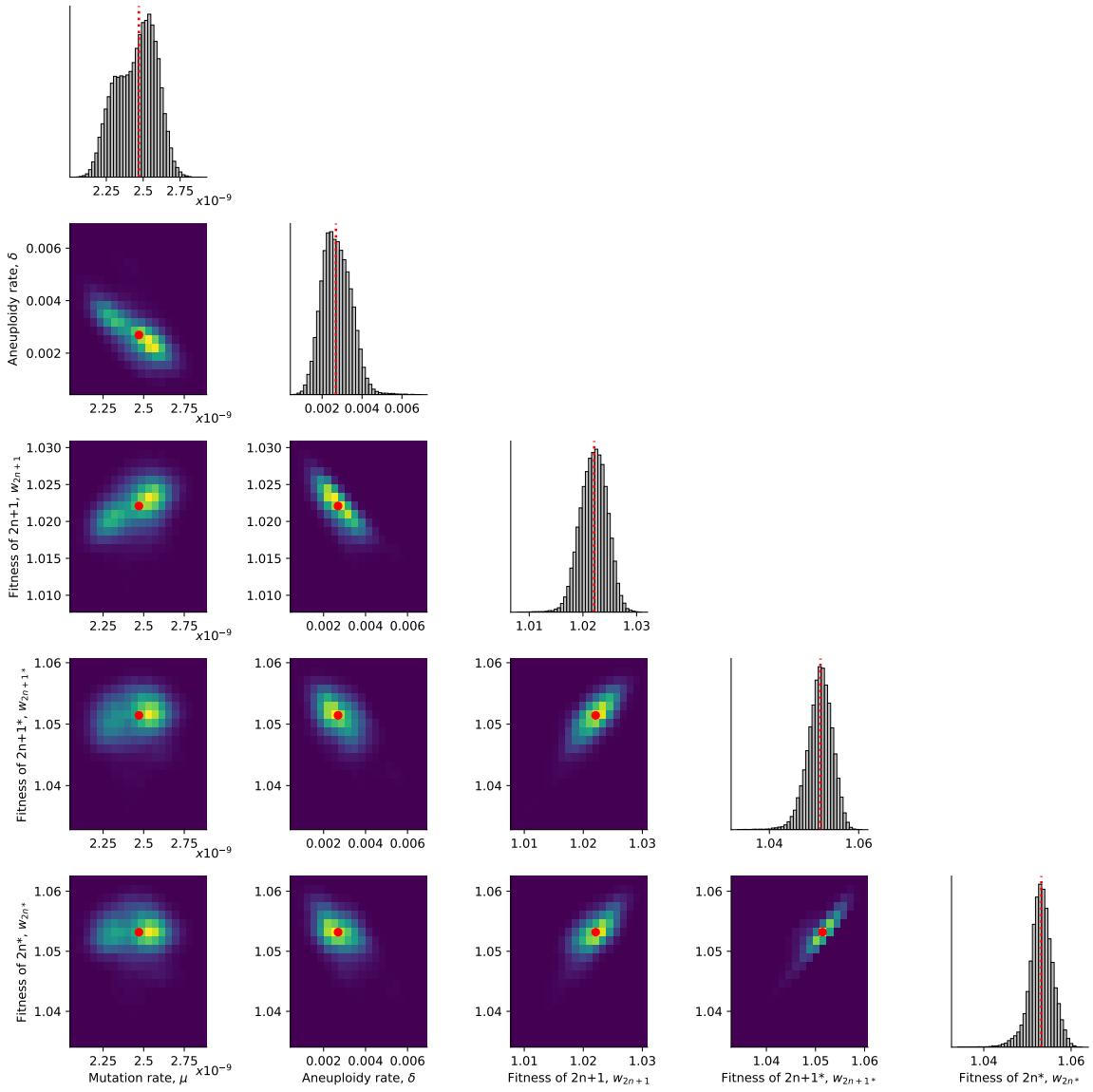
WAIC defined in eq. (6).



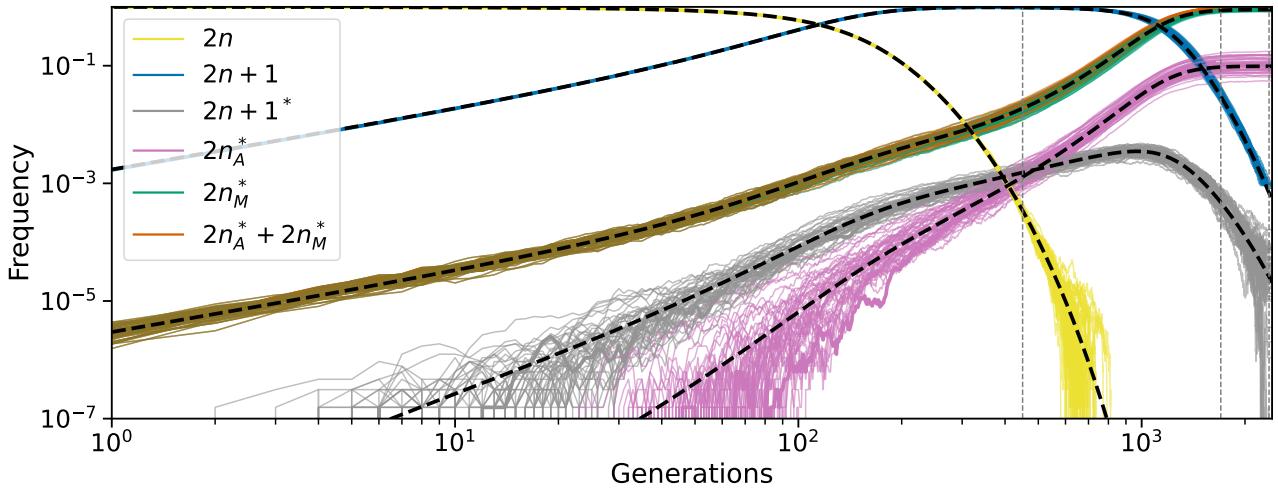
**Figure S6: Likelihood profiles.** Sensitivity of the model approximate likelihood,  $\mathcal{L}(\theta)$ , to changing a single parameter while the other parameters remain fixed at their MAP estimates. Dashed vertical line represents the MAP value. The prior distributions for the mutation rate and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



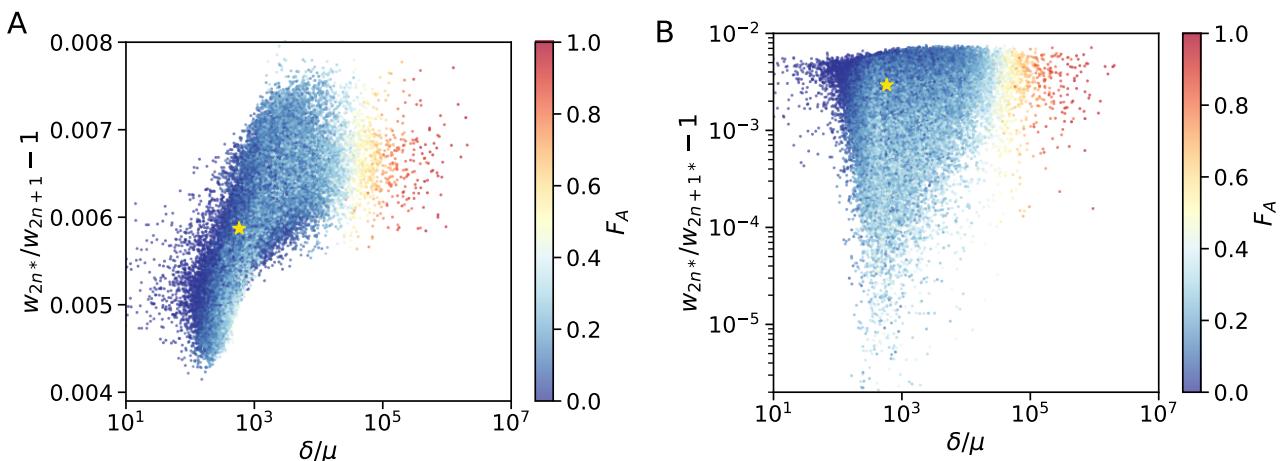
**Figure S7: Model with fixed mutation rate.** (A-D) The inferred posterior distributions for models with free and fixed mutation rate,  $\mu$ . The MAP (maximum a posteriori) and 50% HDI (highest density interval) for each model are: **free  $\mu$ , run 1:**  $\delta = 1.720 \cdot 10^{-3}$  [ $1.470 \cdot 10^{-3} - 2.786 \cdot 10^{-3}$ ],  $w_{2n+1} = 1.022$  [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.