

Sex alters molecular evolution in diploid experimental populations of *S. cerevisiae*

Jun-Yi Leu^{1,4}, Shang-Lin Chang^{1,2,4}, Jung-Chi Chao¹, Laura C. Woods³ and Michael J. McDonald^{1,3*}

Sex is common among eukaryotes, but entails considerable costs. The selective conditions that drive the evolutionary maintenance of sexual reproduction remain an open question. One long-standing explanation is that sex and recombination facilitate adaptation to fluctuating environmental conditions, although the genetic mechanisms that underlie such a benefit have not been empirically observed. In this study, we compare the dynamics and fitness effects of mutations in sexual and asexual diploid populations of the yeast *Saccharomyces cerevisiae* during adaptation to a fluctuating environment. While we find no detectable difference in the rate of adaptation between sexual and asexual populations, only the former evolve high fitness mutations in parallel, a genetic signature of adaptation. Using genetic reconstructions and fitness assays, we demonstrate that evolved, overdominant mutations can be beneficial in asexual populations, but maintained at lower frequencies in sexual populations due to segregation load. Overall these data show that sex alters the molecular basis of adaptation in diploids, and confers both costs and benefits.

Genetic exchange and recombination are almost ubiquitous across eukaryotes. A long-standing question in biology concerns the evolutionary forces that caused sexual reproduction to be so widespread despite its obvious costs¹. A range of explanations have been proposed² that generally focus on two questions. How does recombination alter the fates of the alleles segregating in a population? And what are the ecological conditions that drive the evolution and maintenance of sexual reproduction?

Theory has proposed mechanisms by which recombination makes natural selection more efficient at sorting beneficial and deleterious mutations². In asexual populations, beneficial mutations compete since they cannot be brought together into the same genetic background. Competitive interference between clones extends the time for a beneficial mutation to fix and causes the loss of some beneficial mutations from the population. The Fisher–Muller class of theories propose that the advantage of sex is that recombination speeds adaptation by uniting beneficial mutations onto the same genetic background^{3–5}. Another target of selection is deleterious mutations. The effects of deleterious mutations in asexual populations can be masked by beneficial mutations, and they can spread by hitch-hiking or Muller’s ratchet^{6–8}. The mutational-stochastic theory proposes that this drag on population fitness is resolved by recombination, which decouples deleterious mutations from beneficial mutations so that natural selection can purge them from the population^{6,8}.

A number of experimental studies have demonstrated the capacity of sex and recombination to speed adaptation in experimental populations of yeast^{9–12}. Many of these studies tested multiple growth conditions, but without knowledge of the mutations that caused adaptation. Instead they used the quality of the environment (that is, how stressful it is) to infer whether beneficial or deleterious mutations were driving the difference between sexual and asexual experimental treatments^{10,12}. In a recent study, time-resolved whole-genome sequencing and genetic reconstructions allowed the unambiguous identification and comparison of the mutations

that underlie adaptation in sexual and asexual populations of yeast. That study provided direct evidence that recombination provides a benefit by resolving both Fisher–Muller and mutational-stochastic effects in adapting populations, at least in yeast. One shortcoming of this work is that it was carried out in haploid populations¹³, while many sexual populations are only transiently haploid and spend most of their life as diploids.

Evidence is mounting that ploidy strongly influences the outcomes of evolution. Asexual diploid populations of yeast fix different mutations and adapt more slowly than haploid populations propagated under the same conditions¹⁴. While a recessive beneficial mutation can spread from rare and fix in a haploid population, a dominant allele is more likely to fix in a diploid population. Moreover, some studies have shown that overdominance, also referred to as heterosis or heterozygote advantage, occurs at an appreciable frequency in yeast evolution experiments¹⁵. Sex is predicted to be costly for an overdominant allele, since 50% of the offspring from a heterozygote mating are homozygotes¹⁶. This fitness cost, or segregation load, could result in sexual populations being at a disadvantage compared to asexual populations.

The evolutionary importance of sex depends on the environment¹. However, some theoretical advantages of sex or recombination are not predicated on environmental change. For instance, recombination could rescue a population from Muller’s ratchet in a uniform environment¹. However, many of the best-known explanations for the evolution and maintenance of sex emphasize environmental variation^{1,17}. For instance, the Capricious¹⁷ and the Tangled Bank theories¹ predict that sex generates variable gametes that may gain advantages across diverse niches in spatially structured environments. Environmental variation over time is the focus of the Red Queen hypothesis¹⁸, which posits that sex and recombination facilitate adaptation in response to biotic selection pressures, such as parasites. The Court Jester hypothesis¹⁹ instead focuses on abiotic environmental variations as a major driving force of evolutionary change. While many theories emphasize the relative importance of

¹Institute of Molecular Biology, Academia Sinica, Nangang, Taiwan. ²Genomics Research Center, Academia Sinica, Nangang, Taiwan. ³School of Biological Sciences, Monash University, Victoria, Monash, Australia. ⁴These authors contributed equally: Jun-Yi Leu, Shang-Lin Chang.
*e-mail: mike.mcdonald@monash.edu

space, time and biotic and abiotic driving forces, the selection pressures supplied by any of these should in many ways lead to similar outcomes in terms of the evolution of sex²⁰.

A complete eco-evolutionary model of the evolution of sex should explain the costs and benefits of recombination, as well as the selective pressures that drive and maintain sex. A proposed connection between the mechanisms of recombination and environmental fluctuations is that fluctuations in the form of selection can generate fluctuations in linkage disequilibria that may favour recombination²¹. In one of the few experimental tests of sex on adaptation to a fluctuating environment, Gray and Goddard found that sex facilitated simultaneous adaptation to multiple environments¹¹. However, that study was limited to phenotypic analyses, and the dynamics and fitness of the mutations that underlie adaptation in these conditions were not studied.

Here we present a study of replicate sexual and asexual populations of diploid *Saccharomyces cerevisiae* adapting to an environment that fluctuates temporally between two extreme environmental conditions. We find that both asexual and sexual treatments improve in fitness over 1,440 generations, but that the genetic causes of adaptation are distinct between the two treatments. We track the trajectories of the mutations that underlie evolution in three populations from each treatment and find that recombination alters the dynamics of beneficial and deleterious mutations. Finally, we carry out fitness assays of individual mutations engineered as either heterozygotes or homozygotes, and determine that alleles that have a high fitness as heterozygotes are able to persist as heterozygotes at high frequency in asexual populations but segregate apart in sexual populations.

Results

To test the effects of sex on the outcomes of adaptation, we used two different yeast genotypes that vary in their capacity to undergo sex and recombination. We engineered *spo11Δ spo13Δ* mutants for the asexual treatment. Diploids of this genotype undergo sporulation without recombination or chromosome assortment. The end products of sporulation are two diploid daughter cells that are almost identical to the parent, similar to a mitotic division²² (see Methods). Yeast containing the wild-type (WT) alleles for *SPO11* and *SPO13* sporulate and mate as normal and were used in the sexual treatment.

Six replicate populations of both sexual and asexual diploid *S. cerevisiae* were propagated in a fluctuating environment that switched between two conditions. The first condition, hereafter referred to as the high treatment, was growth at 38.5 °C in nutrient-rich growth media (YPD) supplemented with 1 M NaCl. This was immediately followed by the low treatment, growth at 18.5 °C in YPD media (Fig. 1 and Supplementary Information). *S. cerevisiae* is typically propagated at 30 °C in the laboratory, and culture in YPD media at 18.5 or 38.5 °C with 1 M NaCl is considered an extreme condition. We measured the doubling time of ancestral strains to be approximately 5 and 4 h in the high and low treatment, respectively, compared to 1.5 h in YPD at 30 °C. Every three full cycles, the lines were subject to sporulation conditions where sexual and asexual treatments differed, with sexuals experiencing selection on ura-, leu- and his- plates to separate mat-a and mat-α haploids (Supplementary Information).

We used competition assays to measure the fitness of our experimental populations after approximately 1,440 generations (24 experimental cycles). We measured a per-cycle selection coefficient of each evolved population in the high environment and then separately in the low environment. We found significant improvements in all of the sexual (s1–s6) and asexual (a1–a6) populations in the high environmental treatment compared to the ancestor (paired *t*-test, Bonferroni corrected $\alpha=0.0041$; Fig. 2a). Interestingly, some asexual and sexual populations decreased in fitness relative to the

ancestor in the low treatment condition (paired *t*-test, Bonferroni corrected $\alpha=0.0041$; Fig. 2b). Previous studies of microbial adaptation have found that low-fitness genotypes adapt faster than high-fitness^{23–25}. Since the ancestor performed worse under high than low conditions, this may explain the greater improvements in the former.

A central claim of any theory of sexual reproduction is that sex and recombination increase the rate of adaptation. We found no difference between asex and sex overall in both the high and low environment, not including the sporulation cycle (high: Mann-Whitney U , $z=-0.08$, $P=0.93$; low: Mann-Whitney U , $z=-0.88$, $P=0.37$; Fig. 2). We also measured the relative fitness of each evolved population for a full cycle through the high- and low-treatment environments. The ancestor strain was not sufficiently fit to persist in co-culture with any evolved population through a full cycle of a fitness assay, so we chose a clone from one of the least fit asexual populations (a1) to use as the labelled reference strain for competitive fitness assays. We found that five sexual populations and three asexual populations had significantly higher fitness than this clone from the asexual population (paired *t*-test, Bonferroni corrected $\alpha=0.0045$; Fig. 2c). Again, there was no overall significant difference between sex and asex (Mann-Whitney U , $z=-0.56$, $P=0.57$; Fig. 2c).

In contrast to several previous studies^{9,12,13}, we did not observe that sex increased the rate of adaptation. To understand the impact of sex on the genetic basis of adaptation in this experiment, we carried out whole-population sequencing after approximately 1,440 generations for all 12 replicate populations. There are two points to note about the frequency of alleles measured using whole-population sequencing in diploid populations. First, because we measure the allele frequency and not the diploid genotype frequency, we do not know the departure from Hardy-Weinberg proportions and cannot distinguish, for example, a population composed entirely of heterozygotes from one with 25% WT/50% heterozygote/25% mutant homozygote. Second, asexual populations do not undergo meiosis and, although relatively rare, loss of heterozygosity events typically affect a large fraction of the genome and can be regularly observed, especially when selection favours homozygotes^{14,26}.

We found that, while sexual populations had similar numbers of mutations, they fixed fewer homozygous mutations than asexual populations (Fig. 2d). This is remarkable since the asexual populations in this study did not undergo meiosis. To compare asexual and sexual populations, we looked at mutations that attained an intermediate frequency compatible with heterozygosity and found that a significantly greater proportion of mutations went to a high frequency in asexual populations (two-proportion *z*-test, $P<1\times 10^{-5}$). This suggests that in sexual populations only beneficial mutations are being promoted by selection, while in the asexual population neutral and deleterious mutations may be able to hitch-hike to high frequencies. This is supported by the sexual populations having a significantly lower frequency of synonymous and intergenic mutations than asexual populations (two-proportion *z*-test, $P=1\times 10^{-4}$). Half of the mutations in asexual populations were synonymous or occurred in intergenic regions, but no synonymous and few intergenic mutations fixed in sexual populations. Synonymous and intergenic mutations are less likely to cause phenotypic change, and these data suggest that those mutations are being decoupled from beneficial mutations by recombination in sexual populations. This is in agreement with a previous study carried out in haploid populations of yeast¹³, and supports the proposal that selection in recombining populations can act on the individual fitness effect of alleles segregating in a population, rather than sets of mutations linked on the same genetic background.

A signature of adaptive evolution is parallel evolution, the evolution of mutations in the same genes across independently evolving populations²⁷. Multiple evolution experiments have found pervasive

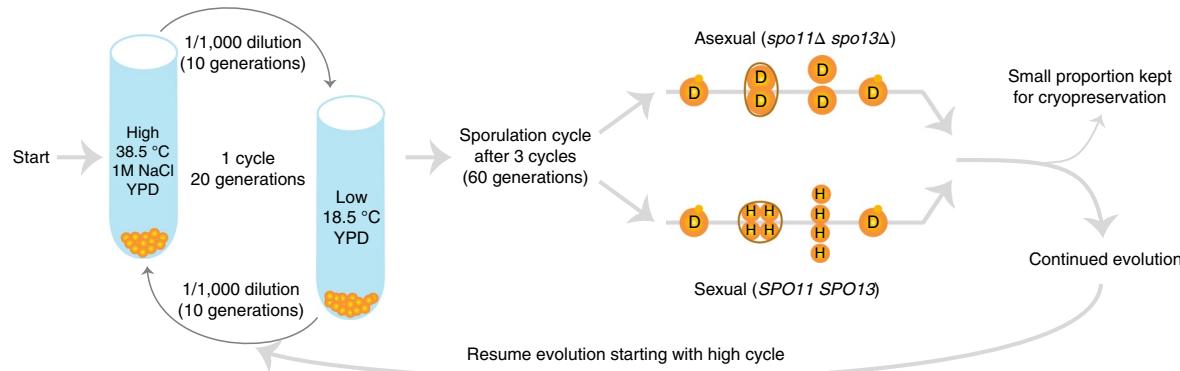


Fig. 1 | Experimental populations of diploids were propagated in a fluctuating environment. After three cycles under both low and high treatment, each diploid population was then sporulated. Note that the asexual populations were *spo11Δ spo13Δ* mutants and that sporulation produced diploid daughter cells identical to the mother cell. Asexual and sexual populations were selected on different growth media (Supplementary Information). Spores from the same population were germinated and densely spread on YPD plates. While diploids from asexual spores would not mate, haploids from the sexual populations mated to form recombinant diploids. All populations were then propagated for another cycle of high and low treatments, and a sample cryopreserved for later analysis. D, diploid; H, haploid.

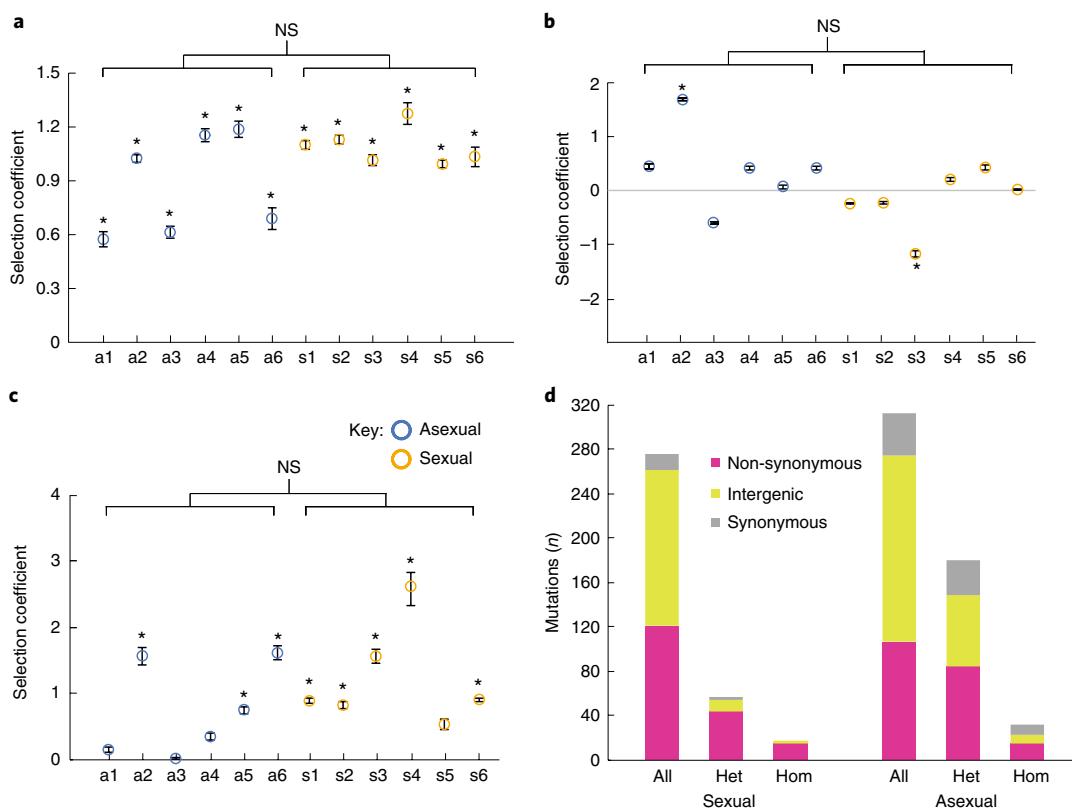


Fig. 2 | Total fitness difference (selection coefficient per cycle) for evolved asexual and sexual populations compared to reference. **a-c**, Competition with the ancestor through one high-treatment cycle (**a**), one low-treatment cycle (**b**) and against a clone from population a1 across a full high/low experimental cycle (**c**). *, Significance <0.01 after Bonferroni correction of P values for t -tests. Between-treatment significance judged by Mann-Whitney U-test. Error bars show ± 1 s.e.m. **d**, Classes of mutations found using whole-population sequencing of all 12 replicate populations. 'All' represents all mutations that exceeded the discovery threshold (see Methods). NS, not significant; het, mutations attaining frequency 0.4–0.9; hom, mutations attaining frequency ≥ 0.9 .

parallel evolution in asexual populations^{28–32}, and genetic parallelism is one of the most repeatable patterns in evolutionary studies across a wide range of systems^{33–35}. To identify the mutations that

drive adaptation in this experiment, we looked for genes that sustained mutations more frequently than expected under a null model. We found that sexual populations had 13 multi-hit genes, including

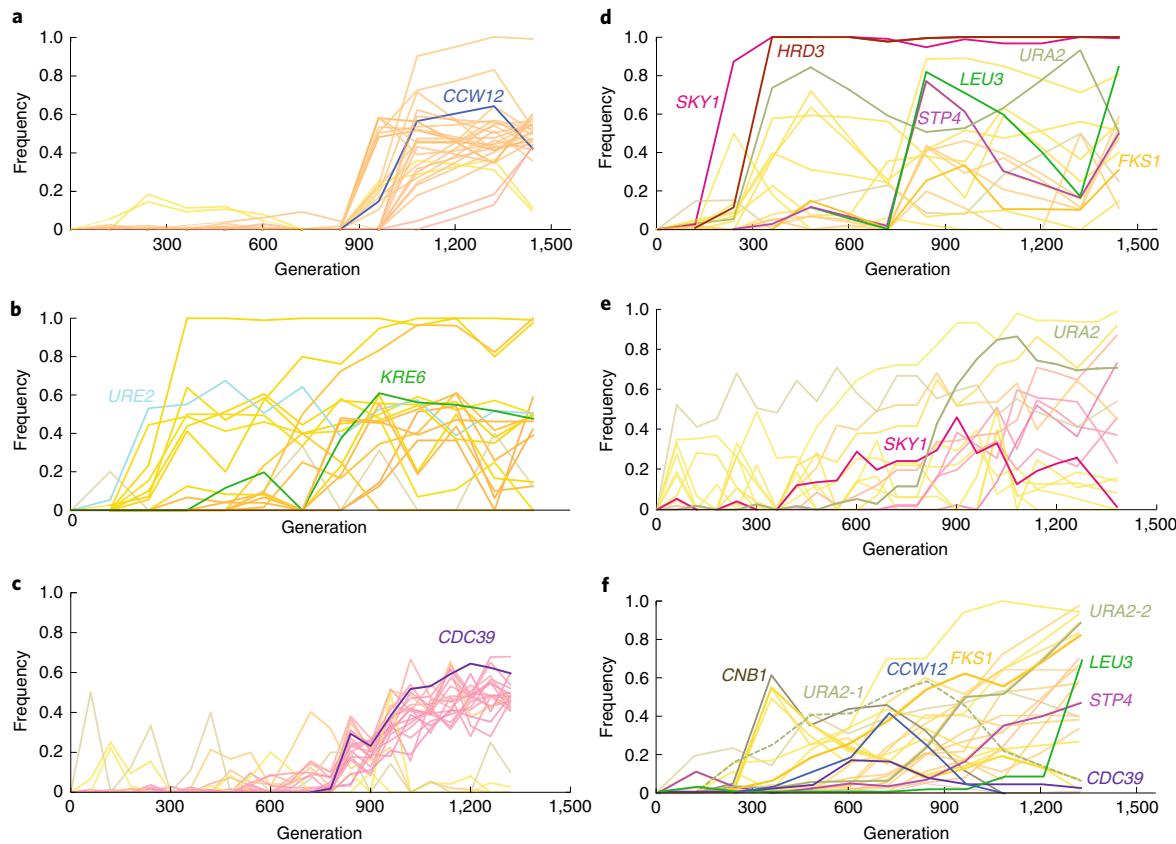


Fig. 3 | The dynamics of mutations in asexual and sexual populations over ~1,440 generations of evolution. **a–f**, Mutations in asexual (a3–5 in a–c, respectively) and sexual (s1, s3, s6 in d–f, respectively) populations. Each line depicts the evolutionary trajectory of a mutation that occurred de novo during evolution. Mutations, except for multi-hit genes, are shaded from yellow to red based on their order of appearance. Multi-hit genes (see Methods) are labelled with gene names shown in the same colour as the corresponding trajectory.

three genes hit three times and one gene hit five times across the six populations, significantly more than expected by chance (simulation test, $P < 10^{-4}$). There were only four multi-hit genes in the asexual populations and none hit more than twice, which was not significantly different from the expectation in populations not experiencing selection (simulation test, $P = 0.58$). Combining data from the asexual and sexual treatments revealed that several of the genes hit only once in the asexual populations (*SKY1*, *HRD3*, *LEU3* and *FKS1*) were multi-hit genes in the sexual populations. This supports the fact that these genes also drove adaptation in the asexual populations, even though they were not multi-hit genes in the asexual populations.

The parallel evolution of mutations in the same genes or pathways in independently evolving populations is the result of deterministic natural selection promoting beneficial mutations. The inconsistency of the outcomes of adaptation at the genetic level in asexual populations suggests that fluctuating environments might impose an additional non-deterministic effect on the dynamics of segregating alleles. We carried out whole-population metagenomics sequencing at regular time intervals for three sexual and three asexual populations so that we could track the fates of the mutations that underlie adaptation (Fig. 3). Asexual populations were characterized by clonal interference and cohorts of mutations that arose and segregated together, consistent with previous studies carried out in haploids^{13,31}. The majority of mutations did not go above a frequency of 0.5. This is expected in asexual diploid populations that cannot readily undergo meiotic recombination to create homozygous diploid versions of alleles segregating in the population. Those mutations that did fix as homozygous diploids in asexual

populations probably arose via balanced loss of heterozygosity events recently observed in a sequence analysis of diploid asexual yeast¹⁴, with about half occurring on the right-hand arm of chromosome XII, a known mitotic recombination hotspot³⁶. It should be noted that since all sequenced time points were sampled after growth in the low environment, there could be regular changes in the frequencies of some alleles that were not detected.

Interestingly, some genes, such as *CDC39* and *CCW12*, carried mutations that were maintained in asexual populations while mutations in these genes were selected against in sexual populations (Fig. 3). We chose these, and several other mutations, from one sexual and one asexual population to be engineered into the ancestral genetic background (Fig. 4a,b). Competitive fitness assays were used to determine the fitness effects of each of these mutations as both heterozygote and homozygote (Supplementary Information), in both environments. We found that the mutations that fixed in asexual populations were neutral or beneficial in one of the environments but deleterious in the other (Fig. 4c). We found that two mutations tested from the asexual line s5 (*HAL9* and *PES4*) were not significantly beneficial in either environment, suggesting that they may have risen in frequency by hitch-hiking or had unmeasured benefits in the sporulation phase of the experiment (Supplementary Information). By contrast, the fitness effects of mutations that went to a high frequency in the sexual population were always, on average, beneficial across both environments (Fig. 4d). All populations experienced greater fitness increases in the high compared to the low environment. It is plausible that a mutation that is beneficial in the high environment could be deleterious in the low environment but has, on average, a beneficial effect.

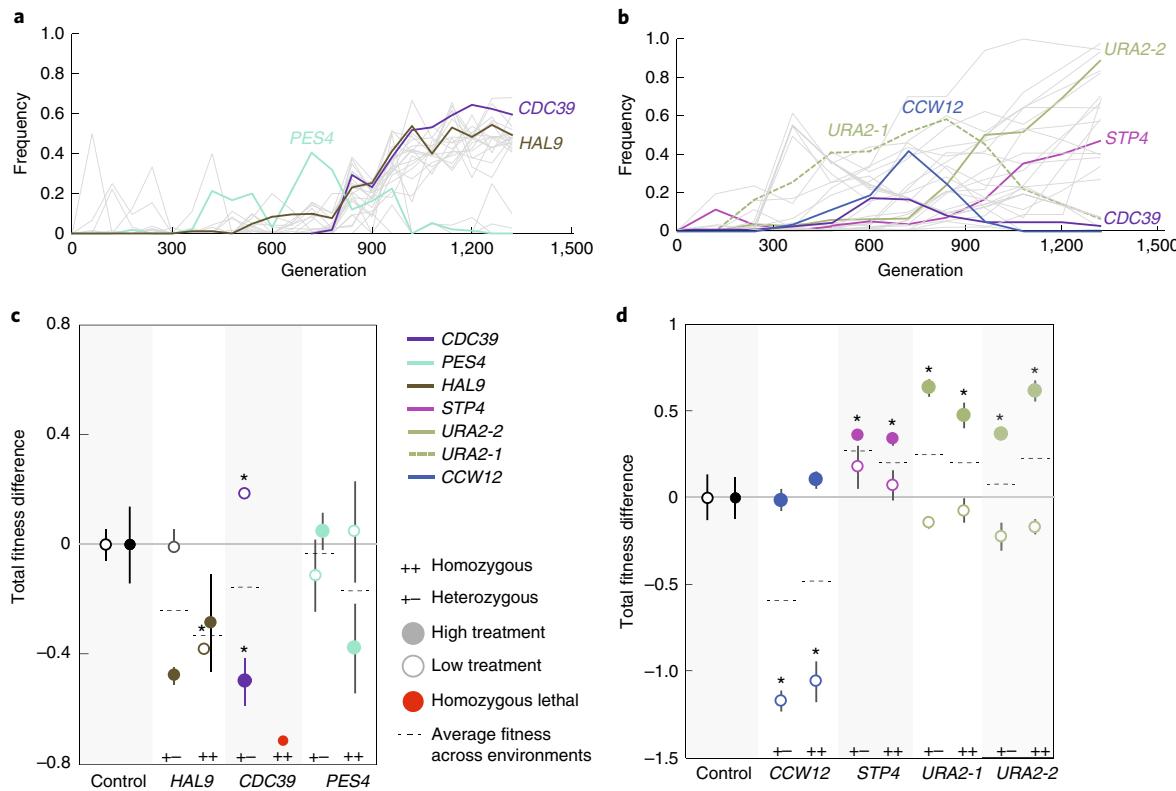


Fig. 4 | The fitness effects of individual mutations from sexual and asexual populations. **a,b**, Trajectories of mutations from asexual population a5 (**a**) and sexual population s6 (**b**). Coloured lines correspond to the trajectories of mutations introduced into the ancestral genotype as a heterozygote and a homozygote. **c,d**, Total fitness difference (selection coefficient per cycle) for mutations from the asexual (**c**) and sexual population (**d**) in the low (open circles) and high (filled circles) environmental conditions, compared to the ancestor. Points with overlapping error bars have been displaced for clarity. Effects of environment and genotype were found in mutations from both asexual and sexual populations; heterozygosity and genotype were found to have significant interactions only in asexual populations (two-way ANOVA; Supplementary Information), and post hoc tests were used to determine significant differences for individual samples. Asterisks indicate significant difference from the ancestor at $P < 0.05$. Error bars show ± 1 s.e.m.

This could explain why some of the populations decreased in fitness in the low environment (Fig. 2).

The molecular basis of adaptation is known to differ between haploids and diploids^{1,37}, with overdominant beneficial mutations proposed to be common in evolving diploid populations^{15,38,39}. Fitness measurements of heterozygotes and homozygotes against a reference strain showed differences in their fitness in the two environments (Fig. 4). We also assayed the capacity for heterozygotes and homozygotes to invade each other by directly competing unlabelled engineered heterozygotes against homozygotes across multiple cycles of the high/low treatments. We found that the homozygotes *HAL9*, *STP4* and *URA2-2* have a significant fitness advantage over their respective heterozygotes. Similar invasion assays confirmed the heterozygote *URA2-1* as being overdominant (Fig. 4 and Supplementary Information). Invasion assays could not be carried out using the *CDC39* allele but, since it was lethal as a homozygote, we could also confirm that the heterozygote mutant is fitter than the homozygote mutant (Fig. 4c). Since the crossing of the overdominant beneficial mutations in heterozygotes results in 50% lower fitness homozygote offspring, sexual populations bear a ‘segregation load’ that is not borne by a non-recombining, asexual population^{16,40}. Consistent with this, a *CDC39* allele established in the sexual population and was maintained at a low frequency as would be expected with balancing selection (Fig. 4).

Our genetic reconstructions also highlight how this segregation load can influence the evolutionary trajectories of mutations in a sexual population. The fitness of two *URA2* alleles was measured

(Fig. 4d), and confirmed using direct competition between unlabelled strains (Supplementary Information). One allele, *URA2-1*, was overdominant while the *URA2-2* allele was fitter as a homozygote. The trajectories of these two alleles (Fig. 4b) are consistent with clonal interference. Clonal interference is possible for these two alleles due to their close proximity on the genome. In an asexual population, clonal interference can be resolved when one of the competing genotypes acquires a new beneficial mutation. In the sexual population, clonal interference can be resolved only by fitness differences of the two alleles, or if a second mutation becomes linked to one of the alleles. Since the fitness of the *URA2-2* homozygote was indistinguishable from that of the *URA2-1* heterozygote (Fig. 4d and Supplementary Information), this suggests that the difference between the alleles in sexual population s6 may have been the segregation load of the *URA2-1* forming less fit homozygotes, allowing *URA2-2* to reach a much higher frequency over the course of the experiment (Fig. 3f).

Discussion

This study presents the dynamics of the mutations that underlie adaptation to fluctuating conditions in recombining diploid populations of yeast. Time-resolved whole-population sequencing has previously shown how recombination increases the efficiency of natural selection by promoting beneficial mutations and purging deleterious mutations in haploid populations of yeast¹³. In agreement with this study, we found that recombination alters the number and composition of the mutations that fix in diploid sexual

populations. Sexual populations fix fewer mutations than asexual mutations overall, with a bias towards non-synonymous mutations. Asexual populations fix more non-synonymous mutations as well as synonymous and intergenic mutations, suggesting that many mutations that are going to a high frequency in asexual populations simply hitch-hike with beneficial mutations. The trajectories of mutations (Fig. 3) revealed that, in asexual populations, alleles increase or decrease in frequency in cohorts. In these populations natural selection acts on sets of mutations that are linked together in fixed genetic backgrounds. In sexual populations, the frequent shuffling of alleles into a large number of combinations allows selection to act on each allele individually.

Despite the observation of parallel evolution in sexual populations, there was no significant difference in the rate of fitness evolution. While it is possible that this is simply due to a lack of statistical power, previous experimental comparisons of sex and asex of similar scale have often found fitness differences^{9,12,13}. Moreover, while we found that asexual populations did not have more multi-hit genes than expected under a null mode, many experiments with asexual populations have shown strong signals of parallel evolution^{28,30,31}. It is worth noting that two of the multi-hit genes in the sexual populations are *URA2* and *LEU3*, involved in the biosynthesis of nutrients leucine and uracil. Selective media without these nutrients were used to select for α and α types of haploid cells, suggesting that part of the difference was that sexual populations experienced stronger selective pressures than asexual populations. Another explanation may be the effect of fluctuating conditions and pleiotropy on asexual populations. Parallel evolution is dependent on the uniformity of selection on a given allele each time it arises by mutation. In asexual populations, natural selection acts on a set of mutations that are linked on the same genetic background^{13,31}. This linkage with a random set of ‘passenger’ mutations will reduce the fixation probabilities of a beneficial mutation³². Such an effect may have been compounded during adaptation to fluctuating conditions by the pleiotropic effects of mutations across the two environments. The possibility that one of the passenger mutations reduces the overall fitness of the genotype will be higher the greater the number of environments in which the genotype is tested.

The DNA sequence data revealed clear differences between sexual and asexual populations. However, the fitness data show that this does not translate into superior fitness outcomes for sexual populations. One explanation for the high fitness of asexual populations is that the lack of recombination allowed cells to maintain the most beneficial genotypic state. The clearest example of this is the loss of function mutations in *CDC39* across multiple asexual and sexual lineages. These mutations fixed as heterozygotes in the asexuals but stayed at a low frequency in the sexual population. This is probably because of the segregation load that arises from the mating of heterozygotes to produce lethal and less-fit homozygotes. This segregation load could also act at other loci showing less extreme forms of overdominance, such as the *URA2* locus. In both cases, these overdominant alleles would be subject to balancing selection in sexual populations so that their overall contribution to population fitness may be reduced relative to sexual populations.

Do fluctuating environments contribute to the maintenance and evolution of sex? While environmental fluctuations may have contributed to weak signals of selection in the genome sequences of asexual populations, experiments with higher replication may be needed to detect differences in the fitness evolution of sexual and asexual populations. Here, the period and regularity of the fluctuations and the order of the high and low environments relative to sporulation experienced by the population may have contributed to some of the results of this study, and studies in a range of variable environments need to be carried out. Altogether, our data show that recombination alters the dynamics and molecular causes of adaptation in diploid populations. The overdominant mutations that we

have characterized and tracked provide rare case studies of experimentally evolved, overdominant mutations in the context of sexual and asexual populations. However, more examples need to be studied before general conclusions can be drawn about how overdominance and sex have evolved, and influenced the other’s evolution.

Methods

Genotype and strain construction. The strains used in this study are based on a diploid W303 yeast strain with the genotype *MATA/MATα, ho STE5pr-URA3, ade2-1, his3Δ::3xHA, leu2Δ::3xHA, trp1-1, can1::MFA1pr-HIS3 MF(ALPHA)1pr-LEU2* (JYL1130 x JYL1129) and *MATA/MATα, ho, STE5pr-URA3, ade2-1, his3Δ::3xHA, leu2Δ::3xHA, trp1-1, can1::MFA1pr-HIS3 MF(ALPHA)1pr-LEU2, spo11Δ, spo13Δ* (JYL1134 x JYL1133). There are several features of these strains that are relevant for this experiment (Supplementary Information). First to note are the nutrient markers that are driven by promoters specific to haploid cells. The *STE5pr-URA3* marker is expressed in haploid cells, but not in diploid cells, facilitating selection for haploids on CSM-ura media and selection for diploids on complete supplement media (CSM) supplemented with 5-fluoroorotic acid (5-FOA). Haploids of mating type α were selected for using the *MFA1pr-HIS3* construct and mating type α using *MF(ALPHA)1pr-LEU2*. The second are the *spo11Δ* and *spo13Δ* deletion mutants that allow for sporulation of diploids, but without meiotic recombination or independent assortment. Briefly, the *spo11Δ* deletion stops recombination and, due to the lack of adhesion between homologous chromosomes, the sister chromatids always migrate to opposite poles. This in turn stops independent assortment. The *spo13Δ* deletion causes a spindle checkpoint delay that leads to spore formation before the second division can occur. The end result of this process is two diploid daughter cells that are an exact copy of the parent, with the same set of chromosomes²², although occasionally the mutant cells may have a few chromosomes segregating as in meiosis. This genotype can be used as a control for experiments comparing sex and asex, since they can be passed through the same sporulation and mating protocols as the sexual populations. Also of note is that the *HO* locus is inactivated by T189A, G223S, L405S and H475L.

Evolution experiment. Twelve replicate populations were founded. The six sexual populations were founded by crosses with JYL1130 x JYL1129 and the six asexual populations by a cross with JYL1134 x JYL1133. All populations were propagated in 15-ml glass tubes containing 3 ml of media. After 48 h of growth, 3 µl of the culture was used to inoculate into 3 ml of media, resulting in a 1,000-fold dilution and approximately ten generations. The minimum population size during this transfer was $\sim 1 \times 10^5$ colony-forming units. All populations alternated between growth in YPD supplemented with 1 M NaCl and incubated at 38.5 °C (high treatment) and growth in YPD media at 18.5 °C (low treatment; Fig. 1) for two rounds (with no sporulation treatment). At the beginning of the experiment the ancestral strains grew slightly too slowly in the high environment for transfer every 48 h, so for the first four transfers were allowed an additional 4–5 h to grow. Every six transfers (three in each treatment), all replicate populations were induced to sporulate by propagation in 3 ml of liquid yeast peptone acetate (10% yeast extract, 2% peptone and 2% potassium acetate) for 12 h, and cells were pelleted and resuspended in the 3 ml of 2% potassium acetate for 3 days. The formation of spores was confirmed by microscopy. Half of the spores were plated on CSM –Ura –His to select for mating type α (*MATA*) and the other half plated on CSM –Ura –Leu to select for mating type α (*MATα*). Cells were washed from each plate, and *MATA* and *MATα* from the same population were resuspended in 50 µl of YPD, combined and spotted onto a YPD agar plate and left for 5 h for mating. To ensure that no diploids had survived the selection for α/α diploids, they were plated onto CSM plates supplemented with 5-FOA. Since haploid individuals express this, URA3 would be unable to grow in the presence of 5-FOA, ensuring minimization of haploid individual that failed to mate (Supplementary Information). The resultant diploids were then used to found another round of exposure to the high- and low-treatment conditions. *MATA/MATα* selection and diploid selection treatments imposed a minimum population size of 1×10^6 CFU.

Fitness assays. Competitive fitness assays were carried out to estimate the fitness of evolved populations and reconstructed mutants. Three days before competition assays were to be carried out, strains were revived overnight from cryopreservation tubes into tubes containing YPD and then into media matching the growth conditions of the competition assay. The competitor strain and a γ -green fluorescent protein (γ -GFP)-labelled ancestral strain were mixed in equal frequencies, and then diluted 1/1,000 into fresh media and incubated for 48 h. The ratio of fluorescent to dark cells was measured using the flow cytometry FACScan system (Becton Dickinson). The gating strategy used to differentiate γ -GFP-labelled from non-fluorescing cells is shown in Supplementary Information. Samples of the mixture both before and after competition were measured, with a minimum of four replicates for every strain, and 30,000 cells analysed for each time point. A selection coefficient was calculated by taking the natural logarithm of the ratio of these measurements.

To measure the competitive fitness in the high/low cycle as in the evolution experiment (Fig. 3c), samples were mixed with a reference asexually evolved clone a1 in a 1/1 ratio of 10^8 cells ml⁻¹. Three microlitres of cell mixture was used and

went through the high/low condition for two rounds. Cells were plated out on YPD plates before and after the cycle to form colonies. Replica plating was used to differentiate the sample and G418-resistant reference strain, and ~500 colonies were counted. A selection coefficient was calculated by taking the natural logarithm of the ratio of these measurements.

Sequencing and variant calling. Cryopreserved populations to be sequenced were fully defrosted, mixed by vortexing and 20 µl inoculated into 3 ml of YPD, and incubated with shaking at 30 °C for 16 h. Genomic DNA was isolated using the Yeastar Genomic DNA kit (Zymo Research). Library preparations were prepared with the Nextera library preparation kit, using a protocol previously described¹¹. Libraries were sequenced to an average 70-fold coverage using an Illumina HiSeq 2500.

We analysed sequence data using a pipeline described previously¹³. Briefly, Illumina reads were trimmed and then aligned using bowtie2 v.2.1.0 (ref. ⁴²). Duplicate reads were marked with Picard v.1.44, and a list of single-nucleotide polymorphisms (SNPs) and indels called using GATK's UnifiedGenotyper v.2.3 (ref. ⁴³). To find low-frequency variants, we set the minimum phred-scaled confidence threshold for GATK required to call a mutation at 4.0. We used the number of reads supporting the reference and alternate alleles from the resulting VCF file to calculate mutation population frequencies. To distinguish between true mutations and errors arising due to library preparation and the DNA sequencing process, we used the following criteria:

1. We excluded mutations with fewer than ten supporting reads.
2. For time course-sequenced populations, we rejected SNPs that did not exceed a frequency of 0.1 for at least two time points.
3. In populations that did not have their full time course sequenced, we excluded mutations that did not exceed a frequency of 0.3.
4. We did not include any mutation that was detected in our resequencing of the ancestor genomes (time point 0).

We annotated each called mutation using a SNP/indel-corrected GFF file and determined its effect on amino acid sequence. Annotations for each SNP or indel were manually confirmed by eye using the Saccharomyces Genome Database (SGD)⁴⁴; these mutations are listed in Supplementary Data 1. For the purpose of analysing mutations that survived natural selection to attain high frequencies, a subset of relatively high-frequency mutations were classified as either het (frequency 0.4–0.9) or hom (>0.9).

It should be noted that we do not estimate genome rearrangements or large indels from changes in depth, since these are difficult to reliably identify and track in whole-population sequence data. Heterozygous fixed alleles are less likely to be called than homozygous fixed alleles, since they are less likely to fulfil the requirement for ten supporting reads and to attain a frequency of 0.1.

Genetic dissection and reconstructions. To measure the fitness effects of individual mutations, we reconstructed mutations from evolved strains in the ancestral genetic background (JYL1129XJYL1130) as both heterozygotes and homozygotes. We used a CRISPR-based strategy adapted from plasmids deposited in addgene by Church Lab⁴⁵. In brief, the guide RNA expression plasmid was modified from pRS426-gRNA-AB, with the URA3 marker being replaced by ADE2. The gRNA expression plasmid was linearized by KpnI and NheI digestion. The guide RNA cassette was amplified by two-fragment fusion PCR using four primers: gRNA-F1 (ACCGTATTACCGCCTTGAG), target gene gRNA-R1 (NnGATCATTTATCTTCACTGCGG), target gene gRNA-F2 (NnGTTTAGAGCTAGAAATAGCAAGTAAAATAAG) and gRNA-R2 (GAAGGGAGAAAGCGAAAGG). Donor DNA sequences were around 400–600 bp in length and were PCR-amplified from the evolved clones. Donor DNA for each allele of interest contained a heterology block⁴⁶ to increase efficiency. The original plasmid carrying the high-specificity Cas9 nuclease, eSpCas9 (1.1)⁴⁷, was obtained from Addgene (plasmid no. 71814) and the Cas9 nuclease was further subcloned into the yeast v141 vector carrying a hygromycin-resistant marker in our laboratory. Each mutation was constructed using the WT Cas9 nuclease, where the Cas9 nuclease was also subcloned into the hygromycin-carrying vector. CRISPR-Cas9-reconstituted clones were screened by PCR with Donor R primer and an allele-specific primer that has the mutation site at the last position of the 3' end and a mismatched nucleotide at the last third position, to decrease the tendency of non-specific annealing.

Multi-hit genes and parallel evolution analysis. We defined multi-hit genes as those that acquired mutations in the open-reading frames of genes in more than one replicate population during the course of the evolution experiment. To generate a conservative estimate of the number of multi-hit genes, we did not include those carrying the same SNP or indel mutation in multiple populations. Also, for the purposes of this analysis, we did not include genes where a single population had acquired multiple mutations in the same gene: these cases were counted as a single hit.

We generated a null model for the expected number of multi-hit genes in our experiment given no natural selection using an in-house python script. Briefly, the NumPy function ‘random.choice’ was used to create a set of 6,600 elements

(genes) whose probability of being selected was weighted by the gene lengths of the 6,600 genes in *S. cerevisiae* (SGD). A number of draws were made from this set (matching the number of called mutations—for example, 127 for asexual populations). The process was repeated 100,000 times, allowing us to calculate 95% confidence intervals around the occurrence of multi-hit genes (Supplementary Information). We judged experimental results to be significantly different from our null expectations if they fell outside this confidence interval.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Raw sequencing reads used to generate the data in Figs. 2–4 have been deposited in GenBank under the Bioproject ID: PRJNA530331. Custom scripts used for the parallel evolution analysis are available at GitHub (<https://github.com/woodlaur189/Parall-expect>).

Received: 24 March 2019; Accepted: 7 January 2020;

Published online: 10 February 2020

References

1. Bell, G. *The Masterpiece of Nature* (Univ. California Press, 1982).
2. Otto, S. P. & Lenormand, T. Resolving the paradox of sex and recombination. *Nat. Rev. Genet.* **3**, 252–261 (2002).
3. Fisher, R. A. *The Genetical Theory of Natural Selection* (Oxford Univ. Press, 1930).
4. Muller, H. Some genetic aspects of sex. *Am. Nat.* **66**, 118–138 (1932).
5. Crow, J. F. & Kimura, M. Evolution in sexual and asexual populations. *Am. Nat.* **99**, 439–450 (1965).
6. Kondrashov, A. S. Deleterious mutations and the evolution of sexual reproduction. *Nature* **336**, 435–440 (1988).
7. Felsenstein, J. The evolutionary advantage of recombination. *Genetics* **78**, 737–756 (1974).
8. Muller, H. J. The relation of recombination to mutational advance. *Mutat. Res.* **1**, 2–9 (1964).
9. Goddard, M. R., Godfray, H. C. & Burt, A. Sex increases the efficacy of natural selection in experimental yeast populations. *Nature* **434**, 636–640 (2005).
10. Gray, J. C. & Goddard, M. R. Sex enhances adaptation by unlinking beneficial from detrimental mutations in experimental yeast populations. *BMC Evol. Biol.* **12**, 43 (2012).
11. Gray, J. C. & Goddard, M. R. Gene-flow between niches facilitates local adaptation in sexual populations. *Ecol. Lett.* **15**, 955–962 (2012).
12. Zeyl, C. & Bell, G. The advantage of sex in evolving yeast populations. *Nature* **388**, 465–468 (1997).
13. McDonald, M. J., Rice, D. P. & Desai, M. M. Sex speeds adaptation by altering the dynamics of molecular evolution. *Nature* **531**, 233–236 (2016).
14. Marad, D. A., Buskirk, S. W. & Lang, G. I. Altered access to beneficial mutations slows adaptation and biases fixed mutations in diploids. *Nat. Ecol. Evol.* **2**, 882–889 (2018).
15. Sellis, D., Kvitek, D. J., Dunn, B., Sherlock, G. & Petrov, D. A. Heterozygote advantage is a common outcome of adaptation in *Saccharomyces cerevisiae*. *Genetics* **203**, 1401–1413 (2016).
16. Lewontin, R. C. & Hubby, J. L. A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. *Genetics* **54**, 595–609 (1966).
17. Maynard-Smith, J. *The Evolution of Sex* (Cambridge Univ. Press, 1978).
18. Van Valen, L. A new evolutionary law. *Evolut. Theory* **1**, 1–30 (1973).
19. Barnosky, A. D. Distinguishing the effects of the Red Queen and Court Jester on miocene mammal evolution in the Northern Rocky Mountains. *J. Vertebr. Paleontol.* **21**, 172–185 (2001).
20. Benton, M. J. The Red Queen and the Court Jester: species diversity and the role of biotic and abiotic factors through time. *Science* **323**, 728–732 (2009).
21. Charlesworth, B. Recombination modification in a fluctuating environment. *Genetics* **83**, 181–195 (1976).
22. Shonn, M. A., McCarroll, R. & Murray, A. W. Spol13 protects meiotic cohesin at centromeres in meiosis I. *Genes Dev.* **16**, 1659–1671 (2002).
23. de Visser, J., Zeyl, C. W., Gerrish, P. J., Blanchard, J. L. & Lenski, R. E. Diminishing returns from mutation supply rate in asexual populations. *Science* **283**, 404–406 (1999).
24. Chou, H. H., Chiu, H. C., Delaney, N. F., Segre, D. & Marx, C. J. Diminishing returns epistasis among beneficial mutations decelerates adaptation. *Science* **332**, 1190–1192 (2011).
25. Kryazhimskiy, S., Rice, D. P., Jerison, E. R. & Desai, M. M. Microbial evolution. Global epistasis makes adaptation predictable despite sequence-level stochasticity. *Science* **344**, 1519–1522 (2014).
26. Mandegar, M. A. & Otto, S. P. Mitotic recombination counteracts the benefits of genetic segregation. *Proc. Biol. Sci. R* **274**, 1301–1307 (2007).

27. Schlüter, D., Clifford, E. A., Nemethy, M. & McKinnon, J. S. Parallel evolution and inheritance of quantitative traits. *Am. Nat.* **163**, 809–822 (2004).
28. McDonald, M. J., Gehrig, S. M., Meintjes, P. L., Zhang, X. X. & Rainey, P. B. Adaptive divergence in experimental populations of *Pseudomonas fluorescens*. IV. Genetic constraints guide evolutionary trajectories in a parallel adaptive radiation. *Genetics* **183**, 1041–1053 (2009).
29. Cooper, T. F., Rozen, D. E. & Lenski, R. E. Parallel changes in gene expression after 20,000 generations of evolution in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **100**, 1072–1077 (2003).
30. Tenaillon, O. et al. The molecular diversity of adaptive convergence. *Science* **335**, 457–461 (2012).
31. Lang, G. I. et al. Pervasive genetic hitchhiking and clonal interference in forty evolving yeast populations. *Nature* **500**, 571–574 (2013).
32. Good, B. H., McDonald, M. J., Barrick, J. E., Lenski, R. E. & Desai, M. M. The dynamics of molecular evolution over 60,000 generations. *Nature* **551**, 45–50 (2017).
33. Wichman, H. A., Badgett, M. R., Scott, L. A., Boulian, C. M. & Bull, J. J. Different trajectories of parallel evolution during viral adaptation. *Science* **285**, 422–424 (1999).
34. Zhang, J. Z. Parallel adaptive origins of digestive RNases in Asian and African leaf monkeys. *Nat. Genet.* **38**, 819–823 (2006).
35. Colosimo, P. F. et al. Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. *Science* **307**, 1928–1933 (2005).
36. Magwene, P. M. et al. Outcrossing, mitotic recombination, and life-history trade-offs shape genome evolution in *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA* **108**, 1987–1992 (2011).
37. Fisher, K. J., Buskirk, S. W., Vignogna, R. C., Marad, D. A. & Lang, G. I. Adaptive genome duplication affects patterns of molecular evolution in *Saccharomyces cerevisiae*. *PLoS Genet.* **14**, e1007396 (2018).
38. Sellis, D., Callahan, B. J., Petrov, D. A. & Messer, P. W. Heterozygote advantage as a natural consequence of adaptation in diploids. *Proc. Natl Acad. Sci. USA* **108**, 20666–20671 (2011).
39. Zeyl, C., Vanderford, T. & Carter, M. An evolutionary advantage of haploidy in large yeast populations. *Science* **299**, 555–558 (2003).
40. Crow, J. F. & Kimura, M. *An Introduction to Population Genetics Theory* (Harper and Row, 1970).
41. Baym, M. et al. Inexpensive multiplexed library preparation for megabase-sized genomes. *PLoS ONE* **10**, e0128036 (2015).
42. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* **9**, 357–359 (2012).
43. DePristo, M. A. et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat. Genet.* **43**, 491–498 (2011).
44. Cherry, J. M. et al. Saccharomyces Genome Database: the genomics resource of budding yeast. *Nucleic Acids Res.* **40**, D700–D705 (2012).
45. DiCarlo, J. E. et al. Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems. *Nucleic Acids Res.* **41**, 4336–4343 (2013).
46. Horwitz, A. A. et al. Efficient multiplexed integration of synergistic alleles and metabolic pathways in yeasts via CRISPR-Cas. *Cell Syst.* **1**, 88–96 (2015).
47. Slaymaker, I. M. et al. Rationally engineered Cas9 nucleases with improved specificity. *Science* **351**, 84–88 (2016).

Acknowledgements

J.-Y.L. was supported by Academia Sinica of Taiwan (grant nos. AS-IA-105-L01 and AS-TP-107-ML06) and the Taiwan Ministry of Science and Technology (grant no. MOST107-2321-B-001-010). M.J.M. was supported by ARC Discovery (grant no. DP180102161) and an ARC Future Fellowship (no. FT170100441).

Author contributions

J.-Y.L., S.-L.C. and M.J.M. conceived and designed the study. S.-L.C., J.-C.C. and M.J.M. carried out experiments. J.-Y.L., S.-L.C., L.C.W. and M.J.M. analysed the data.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41559-020-1101-1>.

Correspondence and requests for materials should be addressed to M.J.M.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2020

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.

Data analysis

We analysed sequence data using a pipeline described previously in McDonald et al, Nature 2016. Software within this pipeline includes bowtie2 v2.1.0, Picard v1.44 and GATK's UnifiedGenotyper v2.3. Custom code was developed for an analysis of parallel evolution and has been made available on GitHub.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Raw sequencing reads used to generate the data in figure panel 2B, figure 3 and figure panels 4 and B have been deposited in the NCBI BioProject database under accession number PRJNA530331 for release upon publication. Custom scripts used for the parallel evolution analysis are available GitHub (<https://github.com/woodlaur189/Parall-expect>).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	This experiment has 12 replicate populations, 6 that adapt to a fluctuating environment with sexual reproduction, and 6 others that are unable to undergo meiosis. we have two kinds of data- whole genome sequence data and fitness assays. The sequence data was analysed using a previously described pipeline. We carried out competitive fitness assays each that had at least 4 independent replicates, and required the counting of 30,000 cells per sample per time point.
Research sample	The samples analysed in this study were all evolved in the lab. All evolved replicates were analyzed.
Sampling strategy	We chose to use 6 replicates for each experimental treatment due to previous experience with experimental evolution. Given that natural selection is strong in the conditions used in this experiment, we expected that, like previous studies, we would see repeatable increases in fitness and the repeated occurrence of beneficial mutation across this number of replicates. We also chose the number of replicates for fitness assays based on previous results that had shown that 4 replicates is enough to generate an error distribution that allows us to distinguish evolved populations from the ancestor, or founding strain.
Data collection	Sequence data was generated by a core sequencing facility. The data was analysed by the corresponding author, who utilised a sequencing analysis pipeline that generated lists of genetic variants. Fitness assays were carried out by Research Assistant Jung-Chi Chao, an author on this study. Ms Chao had
Timing and spatial scale	The sequencing data was collected across 2016 and 2017 over two sequencing runs. The fitness data was collected in 2016 for the evolved strains and then in 2018 for the engineered reconstructed mutants.
Data exclusions	During the calling of genetic variants, the sequencing pipeline specifies that the criteria for the genetic variants that are included in the analysis. These criteria are stringent, are described in detail in the methods and follow previous work.
Reproducibility	Fitness assays were repeated for the following reasons: If one or more of the samples had replicates that had low counts, if one or more replicates/strains failed to grow, or if the spread of replicate measurements exceeded 5%.
Randomization	Fitness assays were divided into groups based on whether they were evolved populations, or engineered clones that were generated later in the project. Fitness assays were carried out in blocks based on the treatment (temperature and salt concentration). If a subset of assays had to be repeated due to the types of failures described above, the ancestor strains were always measured again together with the repeat assays to account for between group differences.
Blinding	All replicate measurements for a given treatment could be carried out in a single experiment, so no randomization was carried out to account for between-block differences. In order to separate the generation of fitness data from analysis, JCC, a research assistant, carried out the fitness assays independently from those who may have formed expectations about their outcomes. Data was submitted to JYL and MJM for analysis.

Did the study involve field work? Yes No

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

- | | |
|-------------------------------------|--|
| n/a | Involved in the study |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Human research participants |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data |

Methods

- | | |
|-------------------------------------|--|
| n/a | Involved in the study |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Cell cultures were diluted in water and then submitted for FACS.

Instrument

BD FACScan (Becton Dickenson)

Software

FloJo. This allows gates to be set, and the data extracted and deposited into an excel spreadsheet for analysis.

Cell population abundance

After gating we required that each time point for each replicated has counted at least 30,000 cells

Gating strategy

In supplementary figure 2, we show how our two cell populations are clearly distinguishable, and the gates we choose very conservative

- Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.