Sexual Recombination and the Power of Natural Selection

William R. Rice* and Adam K. Chippindale

Theory predicts that recombination will increase the effectiveness of natural selection. A *Drosophila melanogaster* model system was developed that increased experimental power with the use of high experimental replication, explicit tracking of individual genes, and high but natural levels of background selection. Each of 34 independent experiments traced the fate of a newly arisen mutation located within genome-wide, synthetic chromosomes that were propagated with or without recombination. An intrinsic advantage to recombination was demonstrated by the finding that the realized strength of selection on new mutations was markedly increased when recombination was present.

There are three major classes of genetic hypotheses for the adaptive significance of recombination (*1*). Nonrecombining populations experience (i) faster accumulation of small-effect deleterious mutations [e.g., Muller's ratchet (*2*, *3*)], (ii) slower accumulation of beneficial mutations [e.g., in the context of adaptation to a changing environment during the Red Queen process (*4*–*14*)], and (iii) an increased deterministic mutational load (*15*–*17*). The experiments described here jointly test the first two classes of hypotheses, which can be unified because both derive from the same phenomenon: recombination reduces the extent to which natural selection on new mutations is diluted by stochastic noise generated by collateral selection on genetic backgrounds.

Binomial sampling error and background selection both generate random change in the frequency of a mutation across generations. The former is an inevitable consequence of finite population size and it causes the frequency of a mutation to change in a manner that is independent of direct selection on the mutation itself. Its magnitude is inversely proportional to population size, N. The latter causes additional noise due to a mutation's chance association with genetic backgrounds of different selective value. Its magnitude is proportional to the genetic standard deviation in genome-wide fitness, $\sigma_{W(\mathrm{gen})}$. The dilution of direct selection on a mutation due to the additional noise generated by background selection can be expressed by a lowered effective population size, N_e, in comparison to the census size N (*3*, *6*).

When recombination is present, mutations move between genetic backgrounds each generation (Fig. 1A), in which case a favored mutation can be fixed and a harmful mutation can be purged, no matter what the original genetic background. The net effect of the stochastic noise generated by background selection rapidly averages out as a mutation samples additional genetic backgrounds (*7*–*14*). Therefore, recombination prevents background selection from strongly diluting direct selection on a mutation; i.e., $N_e \approx N$ for feasible levels of background selection (*14*).

When recombination is absent, mutations are trapped in their original genetic background [background trapping; see (*7*–*14*)]. In this case, the effect of the genetic background on organismal fitness persists and interferes with direct selection on a mutation. The only mutations that will ultimately be fixed in a population are those that originated by chance in genotypes whose fitness, which results from the combined effects of the new mutation and the background, is high (*7*–*14*); i.e., they must originate in the "Progenitor tail" shown in Fig. 1B. All other mutations are eliminated deterministically because they are trapped in lower-fitness lineages (genetic backgrounds) that are destined to eventual extinction (the "Living Dead," Fig. 1B) (*10*).

The effective population size (N_e) of a nonrecombining population is the size of the progenitor tail (N_p). The explicit value of N_p depends on the strength of selection on a mutation; e.g., the smaller the selection coefficient of a beneficial mutation, the higher the requisite fitness of its original genetic background to prevent it from being trapped in the living dead (Fig. 1B). N_p will be a very small proportion of the census size ($N_e \ll N$) when (i) the genetic standard deviation for genome-wide fitness is large relative to a mutation's selection coefficient, s, and (ii) there is a large class of mutations with very small effect size. The second condition makes the distribution of fitness nearly continuous and the number of individuals in the progenitor tail small regardless of total population size (*13*). Both of these conditions are realistic for natural populations of multicellular organisms (*18*–*21*).

Overall, theory predicts that recombination increases the realized strength of selection because it prevents background-trapping and thereby reduces the dilution of direct selection by background selection. This prediction can be tested equally well in the context of the accumulation of beneficial mutations or the removal of deleterious mutations because both are a consequence of the same phenomenon—reduced interference between direct versus background selection. Here, we experimentally compare the rates of accumulation of beneficial mutations located on recombining versus nonrecombining chromosomes.

When is recombination favored? In designing an experiment to test the adaptive significance of recombination, it is important to recognize that theory predicts that recombination will influence the rate of fixation of mutations only within a specified window of selection coefficients, s, approximated by $1/N < |s| \ll 6\sigma_{W(\mathrm{gen})}$. Below the lower bound of $1/N$, selection is overpowered by binomial sampling error and mutations freely drift to fixation or loss regardless of recombination (*22*–*24*). The upper bound of the window is set by the range in the distribution of genotypic fitness values, which is approximated by $6\sigma_{W(\mathrm{gen})}$. Only when $|s|$ is small relative to the range in fitness will most mutations be trapped in the living dead and will the advantage to recombination be substantial (Fig. 1B). Empirical data on selection coefficients (*18*, *19*) and standing genetic variation for fitness (*20*, *21*) suggest that a substantial proportion of spontaneous mutations will fall within this window in natural populations, but this will not be the case for the mutations studied in many laboratory experiments.

The model system. Past attempts to measure the effect of recombination on the power of selection have been highly inconsistent (*25*–*38*). Collectively, these experiments had three major limitations. First, most of these experiments did not track the fate of individual mutations but instead treated the genome as a black box and measured the change in fitness over time (or the mean value of an arbitrary quantitative trait) in recombining versus nonrecombining populations. In such studies, the explicit genetic outcome of the experiment was unknown and only a coarse, indirect assessment of theory was possible. Second, in most cases statistical power was limited by low experimental replication (two to three replicates per treatment). Third, and most importantly, all past experiments used strong artificial selection in combination with model systems that had small genome sizes (e.g., yeast, bacteria, or viruses) or consisted of inbred laboratory stocks. Both of these contexts lead to small standing $\sigma^2_{W(\mathrm{gen})}$, and, hence, low levels of background selection.

The strong artificial selection, relative to the low standing genetic variance in fitness, used in

Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA 93106–9610, USA.

*To whom correspondence should be addressed. E-mail: rice@lifesci.ucsb.edu

previous laboratory experiments did not ensure that the constraint $1/N < |s| \ll 6\sigma_{W(\text{gen})}$ was met and, hence, could have led to inconsistent results concerning the effect of recombination on selection response. In our experiments, we emulated feasible natural conditions by using moderate levels of selection relative to population size and background selection, and we experimentally verified that the constraint $1/N < s \ll 6\sigma_{W(\text{gen})}$ was met. Thus, there was a strong theoretical prediction that recombination would substantially increase the realized strength of selection.

To test the hypothesis that recombination increases the realized strength of selection, we performed 34 independent experiments with a *D. melanogaster* model system. The large number of experiments (17 experiments with and 17 without recombination) was used because we needed to evaluate the average experimental outcome rather than that of a single experiment. Each experiment focused on a beneficial mutation (i.e., one favored by artificial selection in our experiments) introduced to a population of chromosomes that were either recombining or nonrecombining. The beneficial mutations were w^+ alleles at the sex-linked "white" locus. The favored w^+ alleles produced a pigmented eye (red) and the disfavored, loss-of-function allele (w^-) produced an unpigmented eye (white). The experiments quantitatively traced the fate of new beneficial w^+ alleles when introduced into a population fixed for the w^- allele.

Selection was produced experimentally by applying artificial selection on w^+ and w^- alleles located on male-limited chromosomes that were either nonrecombining (synthetic Y) or recombining (synthetic X). We used the large, outbred base population (LH_M) described in (*21*, *39*). To make certain that background selection was substantial (i.e., at least half as strong as it would be in an asexual fly), thereby ensuring statistical power in detecting a recombination–no-recombination effect, nearly entire genomic haplotypes (including all of the chromosomes except the dot fourth chromosome that constitutes <1% of the genome) were made to co-segregate like giant, nonrecombining, male-limited Y chromosomes in the synthetic-Y treatment (Fig. 2A). In the synthetic-X treatment, 80% of the genome was free to recombine via segregation of the autosomes (Fig. 2B) (male *D. melanogaster* lacks chromosomal crossing-over and recombines only via segregation of intact chromosomes).

Mutation ($w^- \rightarrow w^+$) was emulated by migration. The w^- allele was first backcrossed 30 times (at least 600 males by 600 females per cross) through the w^+-base population to form a replica of the w^+-base population (the w^- backcross population). This extensive backcrossing placed the w^+ and w^- alleles into nearly identical genetic backgrounds. Migration of a male from the w^+ base population into the w^- backcross population was used as a surrogate for mutation. The migrant male carried a single hemizygous w^+ allele that was functionally identical to a previously newly arisen mutation located within a random genetic background.

In nature, new beneficial mutations start out at frequency of $p = 1/(2N)$, and because of this initial low frequency, almost all are rapidly lost due to binomial sampling error and background selection. Only the influence of background selection is modified by the

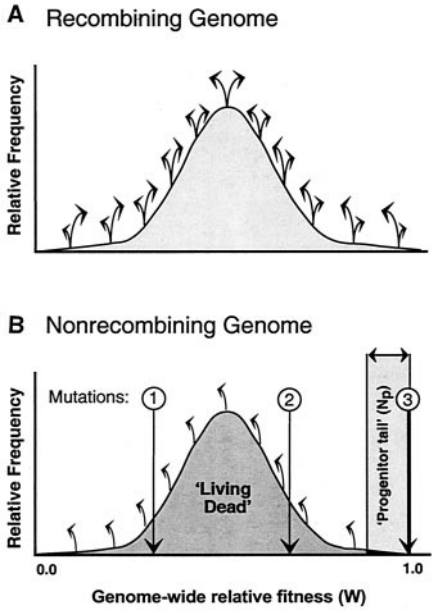

Fig. 1. (**A**) Each generation recombination moves mutations bidirectionally to genetic backgrounds with higher or lower fitness so that new mutations can fix irrespective of their original genetic background. (**B**) In nonrecombining genomes mutations move unidirectionally from better to worse genetic backgrounds (genetic polarization) because most are trapped in low-fitness genomes that are not self-sustaining and experience a net accumulation of deleterious mutations (*10*, *17*). Only the minority of mutations that originate by chance in genomes with highest fitness ("Progenitor tail") can potentially accumulate to fixation (e.g., mutation 3); all others are trapped in low-fitness genomes ("Living Dead") that are not self-sustaining and are thereby destined to eventual loss (e.g., mutations 1 and 2).

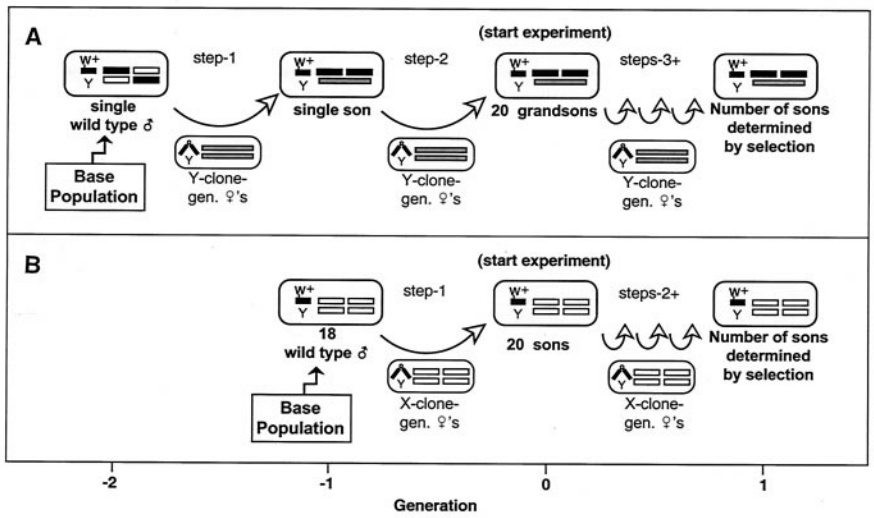

Fig 2. Protocol used to propagate the w^+ alleles in genome-wide synthetic chromosomes. Individual chromosomes are depicted by rectangles [chromosome I = X/Y (left), autosome II (middle), autosome III (right), and the dot chromosome IV is not shown]. Autosomal translocations (which are homozygous female-fertile) are depicted as elongated rectangles, clonally propagated chromosomes are shaded, and chromosomes that are randomly derived from the base population are open. For clarity, genotypes produced from a cross but not retained are not shown. (**A**) To begin the nonrecombining, synthetic-Y treatment a single male was sampled from the base population. He was mated (step-1) to 12 Y-clone generator females (containing an attached X and a translocation of the two major autosomes) (*21*). A single son was retained from this cross which carried an X and two random autosomes from the original male. Next the son was mated (step-2) to 12 Y-clone generator females to produce 20 grandsons, each carrying a copy of the w^+ allele in the same random genomic haplotype. These 20 grandsons were used to begin the selection experiment in generation 0 (Fig. 3A). During the selection experiment genomic haplotypes were clonally transmitted father-to-son by crossing males to virgin Y-clone generator females each generation (steps-3+). (**B**) To begin the recombining, synthetic-X treatment 18 males were sampled from the base population and crossed (step-1) to 18 X-clone generator females [containing an attached X and normal autosomes derived from the base population (*43*)]. Twenty sons from this mating were used to start the selection experiment in generation 0; each carried the w^+ allele in a different genetic background. By propagating males with X-clone generator females during the selection experiment (steps-2+), the autosomes (80% of the genome) were free to recombine via segregation.

presence or absence of recombination. To increase statistical power in detecting the effect of recombination in determining the fate of a new mutation, each experiment started with 20 copies of the mutation. The influence of binomial sampling error in determining the fate of a new mutation was greatly reduced, whereas the influence of genetic backgrounds remained unchanged. This procedure permitted a small number of experiments to efficiently estimate the average outcome of a much larger number that began with a single mutation.

In the nonrecombining synthetic-Y treatment, the beneficial w^+ allele (20 copies, each in the same cloned, random genomic haplotype) (Figs. 2A and 3A) was introduced into a population of 80 w^- alleles (each in a different random genomic haplotype) (Fig. 3A). The beneficial w^+ allele was trapped in its original genetic background, while the w^- allele sampled 80 different genetic backgrounds. In the recombining synthetic-X treatment, 80% of each synthetic chromosome was able to recombine each generation via segregation of the autosomes (Fig. 2B). To ameliorate background-trapping in the remaining 20% of the synthetic-X that was clonally propagated, the favored w^+ allele was introduced in 20 different genetic backgrounds at the start of each experiment (Figs. 2B and 3B). This procedure increased the diversity of backgrounds sampled by the favored w^+ allele by emulating the reduced background trapping that occurs when recombination is present. The nonrecombining, synthetic-Y treatment caused a beneficial w^+ allele to be trapped in its original random genetic background whereas the recombining synthetic-X treatment produced minimal background-trapping. Because of the high starting frequency ($p = 0.2$ instead of 0.01), the fate of the w^+ allele was determined primarily by the relative efficiency of selection, with and without recombination, rather than by binomial sampling error.

To control the strength of selection on the w^+ allele, we first created an environment that removed natural selection against the w^- allele, and then applied an experimentally controlled level of positive artificial selection to the w^+ allele. Previous work by our lab (*40*) and others (*41*) demonstrated that the w^- allele reduces male fitness in two ways: (i) it slows development by about 0.5 days (when reared at 25°C), and (ii) it severely reduces (by ~90%) competitive male mating success under lighted conditions due to blindness of males expressing the w^- allele. Natural selection against the w^- allele was removed by collecting males to begin each subsequent generation late in the 14-day generation cycle (i.e., on day 12.5, when virtually all red- and white-eyed flies had emerged as adults) and by mating flies in the dark so that vision would not influence mating success (in a natural setting, *D. melanogaster* mates both in the dark and in the light). To remove light during mating, each culture vial was housed within a container that allows no light in (a cardboard mailing-tube) while adults were present. Preliminary experiments confirmed that this protocol eliminated all detectable disadvantages of the w^- allele (*40*). To give the red-eyed males a prescribed 10% advantage via artificial selection, we augmented their numbers in each generation by adding 10% extra red-eyed males (from surplus red-eyed males taken from the culture vials that were used to propagate each individual experiment) when males and virgin females were combined to begin each subsequent generation (Fig. 3).

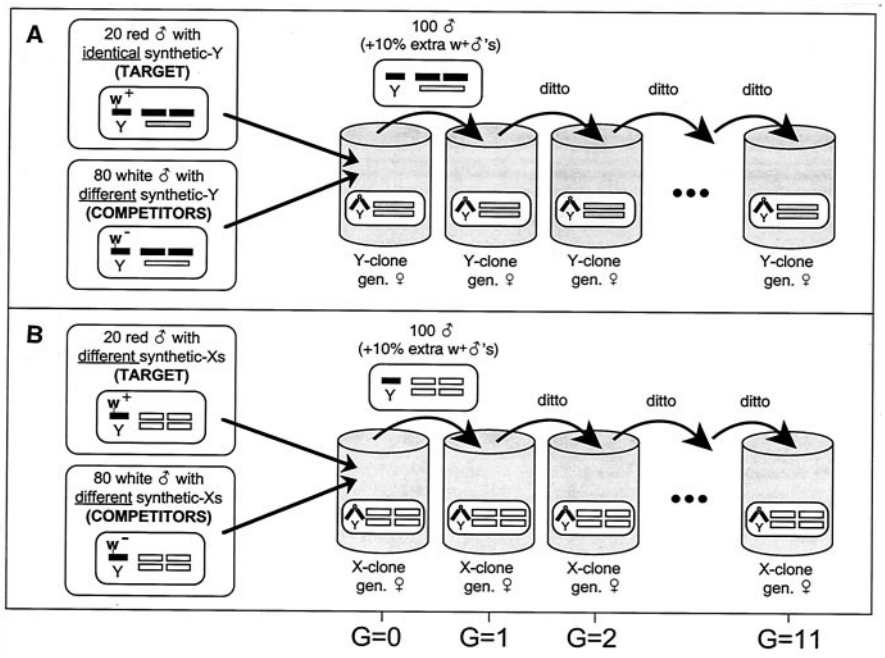

Fig. 3. Protocol for the selection experiment (symbols are as in Fig. 3). (**A**) High background-trapping (synthetic-Y; 0% recombination) treatment. A single genomic haplotype (TARGET) carrying the w^+ allele was cytogenetically cloned into 20 copies (Fig. 2A, steps 1-2). In the same way, 80 competitor males were produced, each carrying a w^- allele in a different genomic haplotype derived from the w^- backcross-population. To begin Generation-0, target and competitor males were combined with 160 virgin Y-clone-generator females, and the females were permitted to lay eggs for 24 hours (*44*). All red-eyed males (carrying w^+ on a synthetic-Y) and white-eyed males (carrying w^- on a synthetic-Y) were collected 12.5 days after egg deposition, counted, and culled to 100 individuals in proportion to their relative numbers. A 10% selective advantage to the w^+ allele was produced by adding 10% extra red-eyed target males to the pool of males that were combined with virgin females at the beginning of each generation. Males were allowed to mature until day 13.5 of the 14-day generation cycle, and then mass-mated in the dark to Y-clone-generator females (taken anew from the stock population) to begin each subsequent generation. This whole process was replicated 17 times to make 17 independent synthetic-Y experiments. (**B**) Low background-trapping treatment (synthetic-X; 80% recombination). The synthetic-X treatment was identical to that of the synthetic-Y treatment except that the w^+ allele was initially in 20 different genetic backgrounds (Fig. 2B, step-1) and X-clone-generator females replace Y-clone-generator females.

Measuring the effect of recombination. The ideal measure of the potential for recombination to increase the power of selection is the rate of fixation of beneficial mutations located on experimental chromosomes with and without recombination. The time to fixation of beneficial mutations, however, is highly variable, and can take hundreds of generations with feasible selection coefficients and natural levels of background selection, even in moderate-sized ($N = 100$) laboratory populations (unpublished computer simulations by W.W.R.). To circumvent this pragmatic obstacle to direct experimentation, we used theory to devise two short-term metrics of the realized strength of selection that were diagnostic of the long-term probability of fixation in recombining versus nonrecombining populations.

First, we estimated the distribution of fitness for the synthetic X and Y chromosomes (i.e., of genomic haplotypes). This allowed us to directly estimate the degree to which natural levels of background trapping would prevent the fixation of beneficial mutations when recombination was absent.

Second, we examined the average dynamic behavior of mutations early in the selection process. When new mutations are initially introduced into a population, the genetic

backgrounds they are associated with are just as likely to be above as they are below median fitness. Therefore, in the early generations, selection on genetic backgrounds, with or without recombination, is just as likely to augment or hinder direct selection on a mutation (*9*). Over subsequent generations, however, a difference between recombining and nonrecombining genomes should become apparent.

In the absence of recombination, most new beneficial mutations are trapped in the living dead (Fig. 1B) when the genetic standard deviation in fitness, $\sigma_{W(\mathrm{gen})}$, is large relative to the selection coefficient s. As a selective sweep of lineages derived from the progenitor tail progresses, the net strength of selection (*42*) declines to zero for beneficial mutations trapped in the living dead, and ultimately becomes negative. In contrast, the net strength of selection for a segregating beneficial mutation is always positive in a recombining population. As a consequence, the realized strength of selection in recombining and nonrecombining genomes can be compared by contrasting the plots of average frequency of the favored w^+ allele ($\bar{p}_{w^+}$, averaged across mutations originating in different random genetic backgrounds) over time when recombination is present versus when it is absent. Early in the experiment, the plots for recombining and nonrecombining genomes should be identical. But over time, a progressing selective sweep by lineages from the progenitor tail (which will eventually lead to loss of the favored w^+ mutations which are trapped in the living dead) will cause the plot for nonrecombining genomes to lag behind that of the recombining genomes.

Requisite strength of background selection. For a strong advantage of recombination to be realized, the constraint $(1/N) < |s| \ll 6\,\sigma_{W(\mathrm{gen})}$ must be met. The constraint that $s > (1/N)$ was satisfied by the experimental design in which (i) at least $N = 100$ adult males were present during the mating competition that occurred at the start of each generation (Fig. 3), and (ii) the advantage to the w^+ allele produced by artificial selection was $s = 0.1$.

To verify the other half of the constraint and to measure the magnitude of background selection attributable to natural fitness variation in a model system, it was required that we estimate the standing genetic variance for fitness [$\sigma^2_{W(\mathrm{gen})}$ among synthetic X and Y chromosomes (i.e., genomic haplotypes)]. This was done by assaying the fitness variation among a large sample of genomic haplotypes from the w^+ base population. The assay began by randomly selecting and cloning 102 genomic haplotypes as described in the synthetic-Y treatment in Fig. 2A. Each cloned genomic haplotype was replicated independently in four separate lines and Δp_{w^+} was measured across one generation, as described in generation 0 of Fig. 3A. Relative fitness was determined by assigning a fitness of 1.0 to the highest fitness genomic haplotype (averaged across all three replicates), and scaling the fitness of all other haplotypes proportionately, as described in (*21*). A random-effects one-way analysis of variance (ANOVA) was used to estimate the genetic variance associated with genomic haplotypes (*21*).

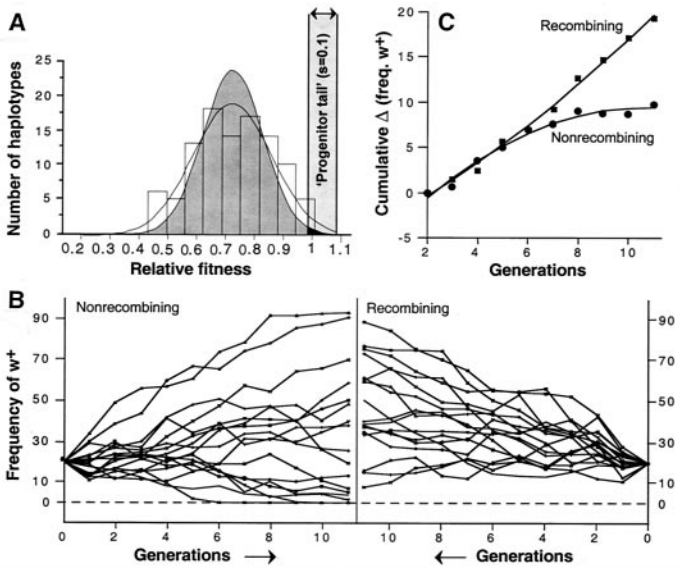

Fig. 4. (**A**) The distribution of net fitness in a sample of 102 genomic haplotypes from the base population. Of the total phenotypic variation among genomic haplotypes shown in the graph (open continuous distribution and histogram), 65% is genetic variation (shaded continuous distribution). The genetic variation in net fitness was substantial [$\sigma_W(\mathrm{gen}) = 0.111$], so that most of the experimental w^+ beneficial mutations ($s = 0.1$) were trapped within the 'Living dead'. (**B**) The frequency of the favored w^+ allele over the course of the experiments in the 17 nonrecombining synthetic-Y populations and the 17 recombining synthetic-X populations. Between generations 0 and 1 no artificial selection was present and fitness was based on solely on background selection. (**C**) The average change in frequency of the favored w^+ allele in the recombining (squares) and nonrecombining (circles) populations. Data are expressed as deviations from the allele frequency in generation 2 (*45*).

Substantial genetic variation for net fitness was found among the sample of 102 genomic haplotypes [$\sigma_{W(\mathrm{gen})} = 0.111$] (Fig. 4A); therefore, with $s = 0.1$ the constraint $|s| \ll 6\sigma_{W(\mathrm{gen})}$ was met. With the observed level of standing genetic variance for fitness, >90% of the favored w^+ mutations would be expected to be trapped in the living dead (background-trapping). As a consequence, most w^+ beneficial mutations eventually would be deterministically eliminated by background selection (Fig. 4A), and a strong advantage to recombination is unequivocally predicted.

Influence of recombination on the power of selection. The large standing genetic variance in the fitness of synthetic-Y chromosomes indicated that chance association with high-fitness versus low-fitness genetic backgrounds should be a major factor in determining whether clonal propagation augments or hinders selection in a particular experiment. When the favored w^+ allele is trapped in an above-average (below-average) genetic background, it will initially increase in frequency faster (slower) than the average case with recombination. In this situation, theory predicts that recombination will have no consistent benefit. As predicted, Fig. 4B illustrates how clonal propagation both accelerated and hindered the rate of accumulation of favored w^+ alleles in different experiments.

Theory makes the consistent prediction, however, that on average the beneficial w^+ allele will eventually accumulate more slowly in nonrecombining genomes, due to background-trapping of most of these beneficial mutations in the living dead. This prediction was met. The plot of cumulative $\Delta\bar{p}_{w^+}$ versus time (Fig. 4C) demonstrates that, on average, the recombining synthetic-X treatments accumulate the w^+ allele at the same rate as the nonrecombining synthetic-Y treatment early in the experiment but that the synthetic-X treatment later surpassed the synthetic-Y treatment. The vectors of average gene frequency versus time diverged with time (Fig. 4C) [intercept and linear terms not significantly different but quadratic terms differed ($F = 5.52$, df = 1,14, $P = 0.00004$)]. The synthetic-X treatment was positively accelerated (quadratic term > 0; $F_{1,7} = 5.76$, $P < 0.05$) and the synthetic-Y treatment was negatively accelerated (quadratic term < 0; $F_{1,7} = 27.75$, $P = 0.001$). By generation 8, the average accumulation of the favored allele was completely arrested in the nonrecombining populations, while during the same period of time the recombining populations continued to rapidly accumulate the favored allele. On average, recombination substantially increased the realized strength of selection.

The major advantage to recombination displayed in Fig. 4C is an inevitable consequence of the high standing genetic variance

observed within the base population (Fig. 4A). Genome-wide fitness variation was estimated to be so large in our experimental assay that mutations with a selection coefficient as large $s = 0.5$ would commonly be trapped in the living dead. This indicates that the proportion of the mutational spectrum whose fixation is promoted by recombination is large. In general, the advantage to recombination demonstrated by these experiments will be manifest in any genome, or genomic subunit, when there is sufficient fitness variation relative to the strength of natural selection, i.e., when a nontrivial proportion of the mutation spectrum will be trapped in the living dead.

Although we did not explicitly examine the accumulation of new harmful mutations, our results reinforce a previous experiment's results (*34*) that demonstrated an accelerated accumulation of harmful mutations in nonrecombining genomes. In general, deleterious mutations fix only when the selection coefficient is weak relative to stochastic noise generated by binomial sampling error and background selection (i.e., $|s| < 1/N_e$) (*22*, *23*). In the absence of recombination a mildly deleterious mutation need only fix by chance within the progenitor tail ($N_p \ll N$) before it will ultimately fix population-wide due to recurrent selective sweeps of the fittest genomes (*13*, *17*). Accordingly, the smaller effective size of a nonrecombining population will cause a wider portion of the spectrum of deleterious mutations to accumulate.

The substantial costs associated with sexual recombination are well established (*1*), making its prevalence in nature an evolutionary enigma. Our results experimentally verify a counteracting advantage of recombining compared to clonal lineages: reduced accumulation of harmful mutations and increased accumulation of beneficial mutations. The magnitude of this benefit will accrue over geological time and promote the superior persistence of recombining lineages at both the level of species within communities (clonal versus sexual species) and genes within chromosomes (nonrecombining Y-linked versus recombining X-linked genes).

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42. The standardized fitness of $w^+ = (\overline{W}_{w^+} - \overline{W})/\overline{W}$, where $\overline{W}_{w^+}$ is the fitness of a w^+ allele averaged over all of its genetic backgrounds, and $\overline{W}$ is mean fitness. This value is related to gene frequency change by, $\Delta p_{w^+} = p_{w^+}(\overline{W}_{w^+} - \overline{W})/\overline{W}$.
43. These autosomes were derived from the base population (depicted as open rectangles). The X-clone generator stock was continuously backcrossed to the LHM base population to maintain genetic diversity of its autosomes.
44. Twice as many Y-clone generator females were used to propagate each generation (160 compared to 80 X-clone generator females) because they produced 50% fewer offspring (due to aneuploid gametes associated with the autosomal translocation). To retain high levels of sexual selection, the 160 Y clone generator females were combined with the males in two groups of 80 virgin females each. The first group of females was combined with the males, and then 4 hours later the second group was added. The 80 virgin females in the synthetic X treatment were combined with the males as a single group. Each generation the total population of flies was evenly distributed among five vials (28.5 mm by 95 mm), with 20 males and 32 females per vial in the synthetic Y treatment, and 20 males and 16 females per vial in the synthetic X treatment. Each vial had a 10-cm extension sleeve attached to its top to provide additional space for pupating juveniles and for courtship among adults.
45. An excess of eggs was laid in the culture vials that were used to propagate the each generation. These were culled to achieve a density matching that to which the base population had adapted for over 200 generations. In generation 1, the vials were not culled sufficiently. At elevated density the w^+ red-eyed flies have a fitness advantage over and beyond that experimentally produced by artificial selection. Accordingly, the first generation was not included in statistical analyses, but the significance of all tests remain unchanged with or without the inclusion of this generation.

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Liver Organogenesis Promoted by Endothelial Cells Prior to Vascular Function

Kunio Matsumoto,[1,2]* Hideyuki Yoshitomi,[1]* Janet Rossant,[1,3] Kenneth S. Zaret[1]†

The embryonic role of endothelial cells and nascent vessels in promoting organogenesis, prior to vascular function, is unclear. We find that early endothelial cells in mouse embryos surround newly specified hepatic endoderm and delimit the mesenchymal domain into which the liver bud grows. In *flk-1* mutant embryos, which lack endothelial cells, hepatic specification occurs, but liver morphogenesis fails prior to mesenchyme invasion. We developed an embryo tissue explant system that permits liver bud vasculogenesis and show that in the absence of endothelial cells, or when the latter are inhibited, there is a selective defect in hepatic outgrowth. We conclude that vasculogenic endothelial cells and nascent vessels are critical for the earliest stages of organogenesis, prior to blood vessel function.

The early stages of visceral organ development serve as a model for changes in cells and tissues that occur in various biological contexts. During the embryonic specification of tissues such as the liver, endodermal epithelial cells receive stimuli from mesodermal cells that cause changes in gene expression and cell division. The endodermal cells differentiate and proliferate within an epithelium, and then begin to move into the surrounding connective tissue. Finally, the cells form a new domain of condensed tissue mass that becomes vascularized. These transitions, which are common to organogensis, tissue regeneration, and tumor growth, require the careful orchestration of signals between epithelial cells, mesenchymal