We demonstrate that fidelity of repair at broken replication forks depends on two partially compensatory mechanisms: cleavage by Mus81 and arrival of a converging fork (Fig. 4E and fig. S11). Converging forks limit the need to reestablish fully functional forks, illustrating an advantage of the multi-origin nature of eukaryotic chromosomes. We propose that deficiencies in Mus81 or timely converging forks may underlie the increased usage of POLD3/Pol32-mediated BIR in cancer cells (*9*) and consequently provide higher adaptation potential to cancer cells and promote tumor progression.

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SUPPLEMENTARY MATERIALS

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EVOLUTION

Fruit flies diversify their offspring in response to parasite infection

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The evolution of sexual reproduction is often explained by Red Queen dynamics: Organisms must continually evolve to maintain fitness relative to interacting organisms, such as parasites. Recombination accompanies sexual reproduction and helps diversify an organism's offspring, so that parasites cannot exploit static host genotypes. Here we show that *Drosophila melanogaster* plastically increases the production of recombinant offspring after infection. The response is consistent across genetic backgrounds, developmental stages, and parasite types but is not induced after sterile wounding. Furthermore, the response appears to be driven by transmission distortion rather than increased recombination. Our study extends the Red Queen model to include the increased production of recombinant offspring and uncovers a remarkable ability of hosts to actively distort their recombination fraction in rapid response to environmental cues.

The first observation that the proportion of recombinant offspring produced by individuals could vary in response to environmental conditions was made in *Drosophila* nearly 100 years ago (*1*). Evidence continues to accumulate that recombination frequency in a variety of species plastically varies in response to factors such as maternal age, temperature, nutritional status, and social stress (*2*–*4*). Theoretical models indicate that plastic recombination can evolve if organismal fitness and recombination frequency are negatively correlated (*5*). Such a negative correlation enables maintaining beneficial combinations of alleles on linked haplotypes while providing opportunities for less fit combinations of alleles to be disrupted and reassembled into potentially more fit haplotypes. Although this fitness-associated recombination model (*5*) appears theoretically tractable for haploids, the model is less applicable for diploids (*6*), because haplotype fitness and organismal fitness are not equivalent. Thus, in spite of numerous observed instances of plastic recombination, a general explanation for its origin and maintenance in natural populations remains elusive.

The evolutionary advantage of sexual reproduction itself, and the independent chromosome segregation and recombination that accompany sex, remain a hotly debated topic in biology. Why cede half of your genetic inheritance to a partner when producing offspring, and shuffle beneficial combinations of alleles that allowed you to survive and reproduce? A leading hypothesis for the evolution of sex is the Red Queen hypothesis, which argues that sex is favored in the face of dynamic selection pressures (*7*, *8*), such as antagonistic interactions with coevolving organisms. Sex and recombination allow parents to diversify their offspring so that competitors, predators, and parasites cannot exploit a static competitor/prey/host genotype. Parasites in particular are thought to be especially important in this process, given that they usually have shorter generation times than their hosts and so can evolve more rapidly. There is strong evidence that host species experiencing parasite-mediated selection pressures are more likely to evolve sexuality (*9*), increased outcrossing (*10*), and increased recombination rates (*11*). There is less evidence that host individuals, during their lifetimes, plastically increase their sexual reproduction (*12*), outcrossing (*13*), or recombination rates in response to parasite threats. In plants, somatic recombination frequency plastically increases in response to immune stress (*14*), and infection leads to a variety of meiotic perturbations, including increased meiotic recombination frequency (*15*). However, there is no direct evidence to date for parasitism-induced plastic increases in meiotic recombination frequency in animals. We used the fruit fly *D. melanogaster* to test whether the proportion of recombinant offspring plastically increases after parasite infection, as predicted by the Red Queen model. We explored parasite-associated plastic recombination using different types of parasites and stresses, in different host life stages, and using different host genetic backgrounds. Further, we began to dissect the mechanism behind the parasite-mediated increase in recombination fraction that we observed.

Borrowing from the rich history of recombination frequency estimation in *D. melanogaster*, we tested whether infection plastically increases the recombination fraction using a classical genetic approach (Fig. 1). We crossed a wild-type strain to a strain with recessive, visible mutations

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in the genes *ebony* and *rough*, which reside approximately 20 centimorgans (cM) apart on chromosome 3R. Female F_1 progeny from this cross are doubly heterozygous, and gametic recombination events occurring between *ebony* and *rough* in these F_1 females were scored after treatment by examining their offspring. To reveal recombination events, the F_1 females were backcrossed to double-mutant males, and backcross 1 individuals were scored: Those carrying a maternal chromosome with a single crossover event between the two markers will have one visible mutation but not the other (Fig. 1).

We first infected virgin F_1 adult females by piercing them in the thorax with a needle dipped in a culture of *Serratia marcescens,* a Gram-negative bacterium that opportunistically infects a wide range of hosts (*16*). We mock-infected a control group with sterile media. The experiment was conducted with four randomly selected wild-type strains from the *Drosophila melanogaster* Genetic Reference Panel (*17*). Four days after treatment, these F_1 females were individually paired with double-mutant males and allowed to mate and oviposit for 5 days. Between 8 and 16 replicate F_1 females were used per treatment per line, which yielded a total of 14,732 progeny scored in the experiment (table S1). The genotype, treatment, and genotype-by-treatment interaction effects on the proportion of recombinant offspring were evaluated using a generalized linear model (*18*). Consistent with the Red Queen hypothesis, treatment explained a significant proportion of the observed variance in recombination fraction ($P = 0.03$, χ^2 test, Fig. 2), with infected flies producing a higher proportion of recombinant offspring than wounded controls. This trend was consistent across all four genetic backgrounds (mean recombination fractions were 0.200 versus 0.178, 0.180 versus 0.178, 0.191 versus 0.187, and 0.199 versus 0.179 for infected versus control flies in lines RAL21, RAL40, RAL45, and RAL75, respectively). No other factors or interactions significantly contributed to the observed variation in recombination fraction in this experiment ($P > 0.35$, all factors, χ^2 test).

The post-infection increase in recombinant offspring we observed may be caused by an increase in meiotic recombination, by an increase in germ cell mitotic recombination, or by some form of transmission distortion, whereby recombinant gametes and/or embryos outnumber or outcompete nonrecombinants (via viability differences among genotypes or asymmetric meiosis where nonrecombinant chromosomes preferentially end up in polar bodies). To test whether the plastic increase in recombination fraction was due to increased mitotic recombination, we conducted a second experiment exploiting the fact that *Drosophila* males do not undergo meiotic recombination (*19*). We infected doubly heterozygous F_1 males with *S. marcescens* and, immediately after treatment, backcrossed these males to double-mutant females. Out of the 9218 flies scored in this experiment, we observed no recombinant progeny. This indicates that there is an overall very low mitotic recombination rate in the *Drosophila* germ line and that bacterial infection does not detectably elevate the rate, at least in males. These data suggest that increased meiotic recombination or transmission distortion, not increased mitotic recombination, are preferred explanations for the increased recombination fraction observed in infected females.

To determine whether transmission distortion contributed to our observations, we relied on the known timing of recombination during *Drosophila* oogenesis: *D. melanogaster* crossovers are initiated and resolved in developing oocytes 4 to 5 days before eggs are fertilized and laid (*20*, *21*). Thus, any increase in the recombination fraction that manifests in the first 4 days after infection must primarily be driven by transmission distortion rather than increased crossing-over during meiotic prophase. We conducted an experiment in which heterozygous females were infected 48 hours after mating and serially transferred to new vials every 4 days for 12 days. Two additional treatments were included in this experiment: a no-treatment control and an infection treatment using a second bacterial species, *Providencia rettgeri*, a Gram-negative bacterium that infects *D. melanogaster* in nature (*22*). Between 120 and 183 replicate females were used per treatment, yielding a total of 32,256 progeny scored in this experiment (table S1). Because these data were significantly overdispersed ($P < 0.001$, Pearson goodness of fit), we used a generalized linear model that includes an overdispersion parameter to test the effects of treatment and time on the mean recombination fraction (*18*). We found a significant effect of treatment ($P = 0.02$, χ^2 test). There was also a significant effect of time ($P = 0.01$, χ^2 test), as expected given that the recombination fraction shows maternal age effects in *Drosophila* (*2*). The interaction between treatment and time was not significant ($P = 0.44$, χ^2 test).

Bolstered by the significant differences in the recombination fraction across treatments, we sought to test predictions of the Red Queen hypothesis using comparisons of means between specific treatment pairs. Consistent with the Red Queen hypothesis, females infected with *S. marcescens* had a significantly higher recombination fraction relative to the sterile wound control in both the 1- to 4-day (mean recombination fractions of 0.211 versus 0.178) and 5- to 12-day (0.195 versus 0.165) collection periods ($P = 0.03$, $P = 0.04$, respectively, two-tailed t test) (Fig. 3). Females infected with *P. rettgeri* showed similar results, with a marginally significant increase in the recombination fraction relative to the sterile wound control in the 1- to 4-day collection period (0.204 versus 0.178; $P = 0.06$, two-tailed t test) and a significant increase in the 5- to 12-day collection period (0.195 versus 0.165; $P = 0.04$, two-tailed t test). The no-treatment control was uninformative in both collection periods, showing no significant difference in recombination fraction relative to the sterile wound or either bacterial treatment ($P > 0.24$, all comparisons). Nonparametric comparisons of means echo these findings (table S2), highlighting the robustness of our results to assumptions regarding the distribution of the error terms. Overall, these data confirm our initial findings of an infection-associated increase in recombination fraction and extend them to a new bacterial parasite. Because this effect manifests in the first 4 days after infection, it is unlikely that the increase in recombination fraction is caused by an increase in the frequency of crossing-over during meiosis; instead, it is probably due to transmission distortion. A rapid increase in the frequency of recombinant progeny consistent with transmission distortion was also observed in *D. melanogaster* in response to heat shock and multiple mating (*23*, *24*). Our data reveal a remarkable ability of hosts to alter their recombination fraction in rapid response to environmental cues.

To determine whether infection by a different kind of parasite, which infects a different host life stage, can also induce a plastic increase in host recombination frequency, we exposed F_1 doubly heterozygous female (Fig. 1) larvae to the parasitic wasp *Leptopilina clavipes*. This wasp lays a single egg in the body cavity of larval flies, which then hatches and consumes the fly from the inside out unless it is melanotically encapsulated and killed by host hemocytes (*25*). Fly larvae that successfully fought off wasp infection were identified in the adult stage by the presence of black capsules in their abdomens. Virgin wasp-infected and control F_1 females were backcrossed to doubly

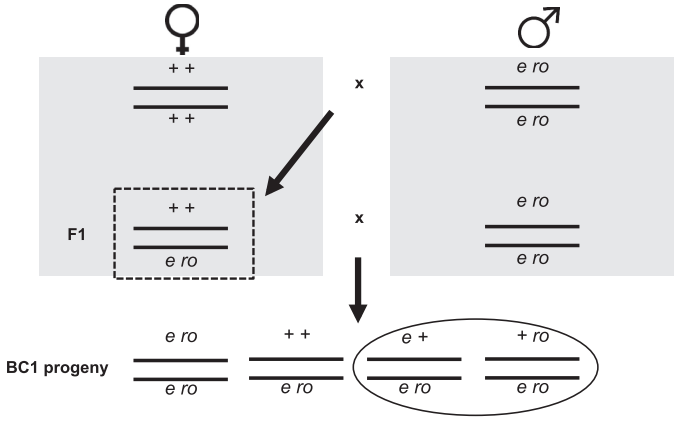

Fig. 1. Schematic representation of the two-step crossing scheme using *ebony* (e) and *rough* (*ro*). Females used in each cross are shown on the left, males on the right. F_1 doubly heterozygous females (dashed box) are those that were subjected to parasite or control treatments. Backcross 1 (BC1) progeny with either of the two recombinant genotypes (circled) can be visually identified using our screen.

marked males (Fig. 1) and were serially transferred into oviposition vials in groups of three individuals every 2 days for 12 days. A total of 87 control and 69 wasp-infection replicates were used in this experiment, which yielded a total of 50,140 progeny (table S1).

We used a generalized linear model to test the effects of treatment and time on the mean recombination fraction. We found a significant effect of treatment (P = 0.0002, χ^2 test), with wasp-infected flies producing a greater proportion of recombinant offspring (0.238) than uninfected controls (0.222). There was no significant effect of time or the interaction between treatment and time (P = 0.15, P = 0.38, respectively, χ^2 test). When analyzed separately for each 2-day egg-laying period, the recombination fraction was significantly increased in wasp-infected flies for the post-mating time periods spanning days 3 to 4 and 5 to 6 (Fig. 4B) (P = 0.02, both comparisons, two-tailed t test). Nonparametric comparisons of means verified these findings (table S2). These data indicate that wasp infection of larval flies, like bacterial infection of adults, leads to a plastic increase in recombinant offspring, once again consistent with the Red Queen model.

This result is surprising given that *Drosophila* larvae contain only primordial ovaries. Ovarioles in *D. melanogaster* females do not begin to develop until after pupariation (*26*); differentiation of the germarial regions (in which crossing-over occurs) within each ovariole takes place in the 24 hours after puparium formation, and synaptonemal complexes (structures required for crossing-over in wild-type *Drosophila*) in the first pro-oocytes become visible in the developing ovaries at 36 hours after puparium formation (*26*). The marked delay between wasp infection and the onset of oocyte formation, coupled with the observation that the recombinational response to the wasp attack is sustained for up to 12 days after mating, shows that the signal underlying the infection-associated increase in recombination fraction can be triggered in the absence of fully developed ovaries and can be sustained across development.

Our data indicate that the proportion of recombinant offspring in *D. melanogaster* plastically increases in response to a variety of parasite pressures. The onset of the response can be both rapid and prolonged, as infected adults increase their recombination fraction within 1 to 4 days after bacterial infection, and wasp-infected larvae develop for several days and undergo metamorphosis before they begin laying eggs at all. Data from both bacteria and wasp-infection trials show that the increased production of recombinant offspring lasts for several days and, for bacterial infection, is significantly stronger than any effect induced by a sterile wound. Because female flies exposed to heat-killed bacteria only show a weak, nonsignificant elevation in recombination fraction relative to wounded flies (0.189 versus 0.178; P = 0.28, two-tailed t test, fig. S1 and table S2), active parasite signals or host immune signaling pathways that specifically respond to live parasites appear to be required for the full-blown recombination response. Furthermore, the

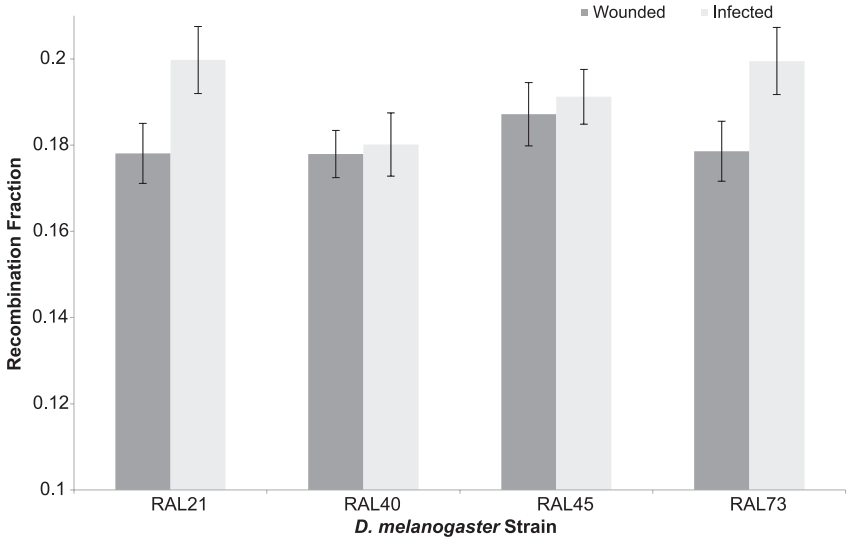

Fig. 2. Recombination fraction for four wild-type strains of *D. melanogaster* mock-infected (wounded) or infected with *S. marcescens*. Error bars represent standard error.

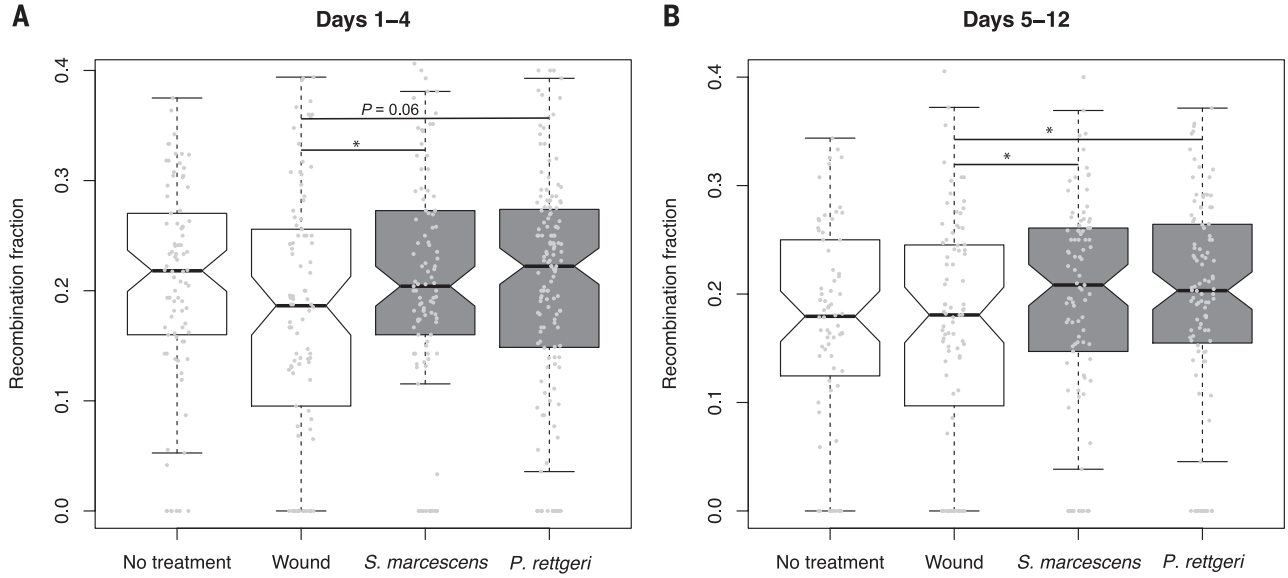

Fig. 3. Box plots illustrating the distribution of recombination fractions in *D. melanogaster* strain RAL73 after one of four treatments: no treatment, sterile wound, infection with *S. marcescens*, or infection with *P. rettgeri*. The median is marked with a black line; the first and third quartiles are represented as the lower and upper edges of the box, respectively. The whiskers extend to the most extreme data point no farther from the box than 1 times the interquartile range. Jittered, individual data points are presented as gray circles. Recombination was estimated separately for eggs laid (**A**) days 1 to 4 after infection and (**B**) days 5 to 12 after infection. Pairwise comparisons of transformed data that are statistically significant based on a two-tailed t test at $P \leq 0.05$ are marked with an asterisk.

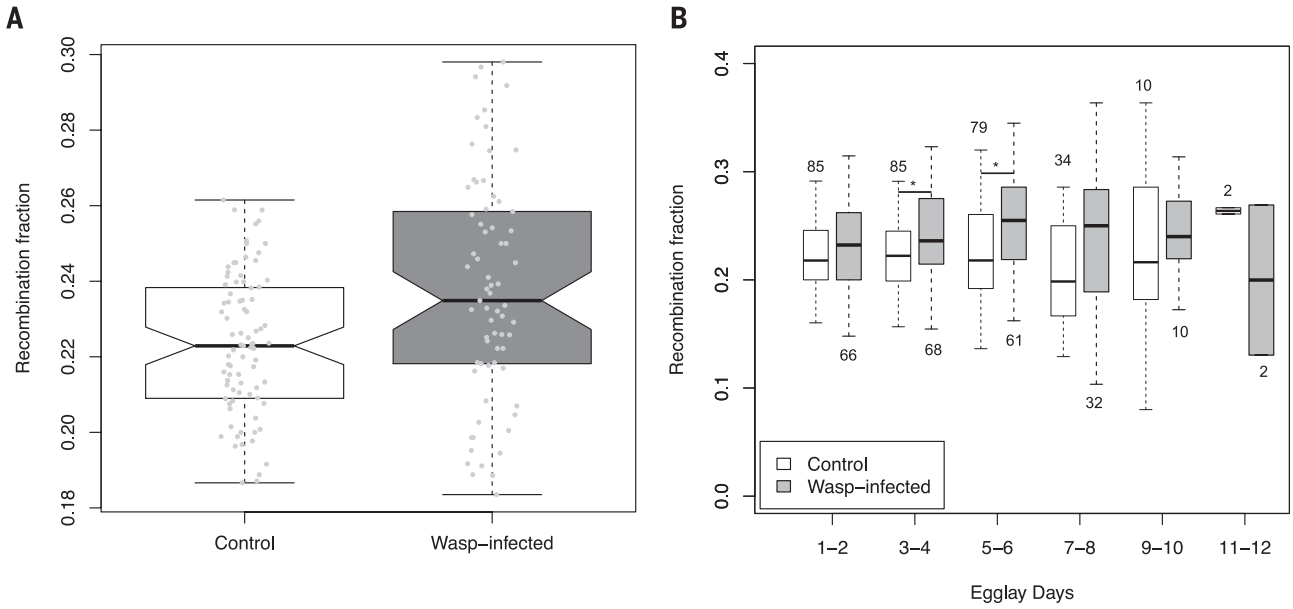

Fig. 4. Box plots illustrating the distribution of recombination fractions in *D. melanogaster* strain RAL73 in control and wasp-infected females. The median is marked with a black line; the first and third quartiles are represented as lower and upper edges of the box, respectively. The whiskers extend to the most extreme data point no farther from the box than 1 times the interquartile range. Recombination fraction is shown (**A**) estimated over the entire 12-day egg-laying period and (**B**) in each of the six 2-day egg-laying periods. In (A), jittered, individual data points are presented as gray circles. In (B), the number of replicates for each time point is included for the control (above the top whisker) and the wasp-infected (below the bottom whisker) treatments. Because there are only two replicates for the 11- to 12-day period, the edges of the box completely span the range of observations. Pairwise comparisons of transformed data that are statistically significant based on a two-tailed *t* test at $P \leq 0.05$ are marked with an asterisk.

increase in recombination fraction is not driven by viability defects caused by an interaction between infection status and the visible markers used in this study (supplementary materials). We find that the mechanism underlying the increase in recombinant offspring is transmission distortion. This distortion could be due to asymmetries during meiosis II or to viability differences between recombinant and nonrecombinant gametes or progeny, and represents an as yet unappreciated mechanism by which *D. melanogaster* females plastically alter the frequency of the recombinant progeny they produce. In the future, it will be important to identify the mechanisms by which this distortion is mediated, as well as determine the extent to which the plastic increase in recombination fraction observed in the current study extends genome-wide, given that previous work has shown that stress-induced changes in recombination frequency are not uniform across the genome (*2*). Overall, our work identifies a strong link between infection and recombination in animals and further extends the Red Queen hypothesis to include plastic changes in recombination in response to environmental stimuli.

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Fruit flies diversify their offspring in response to parasite infection

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