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MATING-INDUCED RECOMBINATION IN FRUIT FLIES

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In traditional deterministic models the conditions for the evolution of sex and sexual behavior are limited because their benefits are context dependent. In novel and adverse environments both multiple mating and recombination can help generate gene combinations that allow for rapid adaptation. Mating frequency often increases in conditions in which recombination might be beneficial; therefore, increased sexual behavior might evolve to act as a cue that stimulates recombination. We conducted two experiments in the fruit fly, *Drosophila melanogaster*, using linked phenotypic markers to determine how recent bouts of additional mating affect female recombination rate. The first experiment examined the effect of additional mating, mating history, and age on female recombination rate. The second experiment assessed the effect of recent mating events on recombination rate. Together, the experiments suggest that each additional bout of mating temporarily increases female recombination rate. These findings imply that the conditions favoring the evolution of sexual reproduction and multiple mating behaviors are broader than currently appreciated.

KEY WORDS: **Cost of mating, cost of sex, *Drosophila melanogaster*, multiple mating, parental effects, recombination, sexual selection.**

The prevalence of sexual reproduction and recombination is one of the long-standing unresolved issues in evolutionary biology (Weismann 1889; Maynard Smith 1978; West et al. 1999). Genes in asexual lineages can potentially spread twice as fast as genes in sexual lineages, causing a twofold cost of sex. In addition, recombination breaks up favorable gene combinations that have increased in frequency by natural selection. Because of these costs, we would expect natural selection to generate mostly asexual lineages, yet we find that sex and recombination are widespread in nature.

Environment-induced recombination, which occurs when environmental factors stimulate recombination, may help explain the maintenance of sex. In novel and adverse environments there may be fitness benefits to breaking up gene combinations that had been favored previously. The costs of sex might be offset if recombination is reduced during times of stasis but increased in novel and adverse environments (Parsons 1988; Hoffman and Parsons 1997; Imasheva 1999; Agrawal et al. 2005; Badyaev 2005). Though in traditional deterministic models, recombination only evolves under a restricted set of conditions (Charlesworth 1993; Kondrashov 1993; Barton 1995), in models that include environment-induced recombination and genetic effects of mothers, the conditions favoring the evolution of recombination are broader (Agrawal et al. 2005).

In the early 1900s a series of experiments revealed that factors such as temperature, nutrition, and age had a profound effect on the recombination rate in fruit flies (Plough 1917; Bridges 1929; Neel 1941). In the years since these studies, temperature, nutrition, age, social interaction, genetic modification, and exposure to antioxidants have all been shown to stimulate recombination in a variety of different systems (reviewed in Parsons 1988; Badyaev 2005). Environmental conditions can also affect the evolution of recombination. For example, chronic exposure to parasites selects for genotypes with increased recombination (Fisher and Schmid-Hempel 2005). And, recombination rate has been found to increase

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as a correlated response to artificial selection (Burt and Bell 1987; Gorodetsky et al. 1990; Korol and Iliadi 1994).

Additional bouts of mating may also stimulate recombination. A possible correlation between mating and recombination is suggested by the effects of maternal age on recombination in fruit flies. In these studies recombination rate fluctuated with age in a consistent pattern (Plough 1917; Bridges 1929; Neel 1941). The initial peak and the subsequent drop in female recombination rate with age were always accompanied by a second peak (Plough 1917; Bridges 1929; Neel 1941; Redfield 1966). Investigators recognized the pattern but its source was not known.

> Certainly the "periodic fluctuations" [in recombination rate] can at this stage of the analysis be as well attributed to the operations of chance and uncontrolled environmental variables as to the effect of some physiological rhythm of the female.
>
> —Neel (1941)

One possible explanation for this pattern is that mating increases recombination rate. The position of the first peak is determined by the initiation of mating (Redfield 1966). The second peak occurs about five or six days after the first peak, close to the average time it takes for *Drosophila melanogaster* females to mate a second time (Singh and Singh 2004). Thus, the recombination rate is highest in the periods immediately after fruit flies are expected to mate.

Effects of mating on recombination rate might have important evolutionary implications. Mating-induced recombination could broaden the conditions for the evolution of sex and allow for the evolution of recombination in a manner analogous to environment-induced recombination. It could also influence the evolution of sexual behavior. It might allow multiple mating females to produce genetically variable offspring through a different mechanism than obtaining sperm from multiple males. Mating-induced recombination might help overcome the costs of sex and the costs of multiple mating.

This paper tests the hypothesis that recent bouts of additional mating can increase the genetic variability of offspring by increasing the crossing over within the female germ line. We conducted two experiments to address this hypothesis using *D. melanogaster*. In the first experiment we measured the recombination rate of females that we observed mating, and assessed the effect of additional mating on recombination rate while accounting for effects of mate history and maternal age. In the second experiment we manipulated male exposure frequency and measured female recombination rate.

Materials and Methods

STOCKS

Strains of *D. melanogaster* with recessive phenotypic markers for the second chromosome, clot (cl^1), black (b^1), purple (pr^1), and cinnabar (cn^1); dominant phenotypic markers for the second chromosome Drop (Dr^1) and Prickled (Pr^1); dominant phenotypic markers for the third chromosome Kruppel (Kr^{If-1}) and Black cell (Bc^1); and a standard wild-type strain, Oregon-R (OR), were obtained from the *Drosophila* stock center in Bloomington, IN. These three sets of markers were chosen because the markers within the intervals were tightly linked, which minimized the occurrence of double recombination events, and were easy to distinguish under a dissecting scope. The cl b pr cn mutations (provided by C. Cronmiller, Biology Dept., UVA), used in experiment 1, were crossed together and isogenized more than 10 years prior to the start of this experiment and were subsequently maintained in laboratory culture. In experiment 2, the genetic background was standardized by backcrossing each marker for eight generations into the Dahomey strain (99.6% Dahomey background). This strain was caught in Dahomey, West Africa , and had been maintained in population cage culture with overlapping generations for more than 30 years prior to the experiment (Partridge and Andrews 1985).

RECOMBINATION ASSAY

In *D. melanogaster*, crossing over between paired chromosomes occurs within the oocytes of females. Similar genetic exchange does not happen in males. To assess recombination in experiment 1, we generated cl b pr cn / + heterozygous females by crossing virgin Oregon-R females to four-day-old virgin cl b pr cn males. Next, we mated cl b pr cn / + females to tester cl b pr cn/ cl b pr cn males and scored the resulting offspring for recombinants and nonrecombinants. The b, pr, and cn markers are closely linked so that recombination events between the markers were rare (15 recombinants every 1000 samples). Double recombinants (which occurred when two recombination events occurred within the cl–cn interval) were very rare (two recombinants every 1000 samples). As a result, we focused our assessment of the recombination rate on the entire cl–cn interval, rather than the recombination rate for each pair of markers in the interval.

In experiment 2, we generated Pr +/+ Dr and Bc +/+ Kr heterozygous females through crossing. We assessed recombination by mating Pr +/+ Dr and Bc +/+ Kr heterozygous females to wild-type Dahomey males and scoring for recombinant and non-recombinant offspring. The recombination distance between the cl–cn, Pr–Dr, and Bc–Kr marker intervals was 38.6, 9.2, and 27.3 cM, respectively.

MATING AND AGE TREATMENTS

In the original studies of recombination rate in fruit flies, the effects of maternal age on recombination rate were confounded by effects of mating (Plough 1917; Bridges 1929; Neel 1941; Redfield 1966). Experiment 1 was designed to assess the effects of maternal age, mating, and mating history. Four different mating treatments were established (Fig. 1A). For the mated-once (eggs

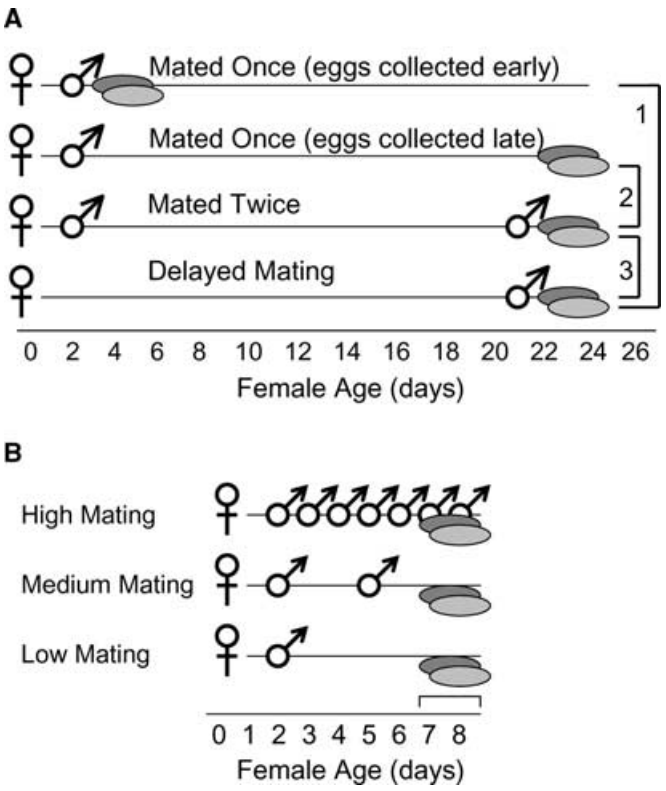

Figure 1. **The mating and egg collection design for (A) experiment 1 and (B) experiment 2. For experiment 1, females heterozygous for phenotypic markers were mated at particular ages to virgin tester males. The male symbol indicates when females were mated. The ovoid shapes indicate when eggs were collected for each treatment. The brackets indicate the comparisons made between treatments to test the effects of (1) maternal age, (2) additional mating, and (3) mating history on recombination. For experiment 2, the symbols describe the mating treatments in the same way except the male symbols now indicate when females were exposed to males for 24 hours.**

collected early) treatment, females were mated once at the age of three days and eggs were collected during the three- to seven-day interval. For the mated-once (eggs collected late) treatment, the females from the previous treatment were held in vials without additional mating and their eggs were collected during the 21- to 26-day interval. For the mated-twice treatment, females were mated at age 3 days and at age 21 days, after which their eggs were collected during the age 21- to 26-day interval. For the delayed-mating treatment, females were held as virgins until they were mated at the age of 21 days and their eggs were collected during the 21- to 26-day interval.

Mating treatments were conducted using tester males and heterozygous females. Cohorts of 100 cl b pr cn virgin tester males were collected every four days for the duration of the experiment. Virgin females that emerged from the OR × cl b pr cn cross were collected every five hours for two days until there were 200 virgin females heterozygous for the phenotypic recessive markers. Females were then randomly assigned mating treatments: 100 for the single and double mating treatments, and 25 females for the delayed-mating treatment (Fig. 1A). For the mated-once and mated-twice mating treatments, the three-day-old females were distributed among four 32-oz. plastic containers (25 females/container) that were smeared with standard molasses-based fruit fly media and had rubber gussets to aspirate copulating flies out of the cage. Forty-two- to four-day-old virgin males were placed in each female cage and the cages were monitored for mating every two minutes. When two flies began to copulate the pair was aspirated out and placed in a single shell vial with media. The male was discarded to prevent remating when the flies broke apart (roughly 18 min later). The duration of copulation was monitored to eliminate pseudo-copulations. After eight hours, 98 of the 100 females had mated one time and the remaining flies were discarded. Thirty of the females were kept for the mated-once treatment, and an early age sample of their eggs was collected over the three- to seven-day interval. The remaining 68 (of 98) flies were kept to mate them a second time (although only 35 were used). When these flies were 21 days old, they were placed in plastic containers that were smeared with fruit fly media and contained a small strip of necrotic banana peel (to stimulate mating). Forty-two- to four-day-old males were placed in the cages and copulating pairs were aspirated as described above. Eighteen females that mated-twice (of 35) were recovered. The same procedure was used to mate the 25 delayed-mating females when they were 21 days old.

During the 21- to 26-day interval, females from the mated-once, mated-twice, and delayed-mating treatments were each placed in fresh shell vials (after minimal CO_2 anesthesia) and the eggs that they laid were collected. After the 21- to 26-day egg collection interval, the adult flies were discarded and the eggs were allowed to develop in the vials they were laid in. The number of females that laid eggs in vials was as follows: 23 for the mated-once treatment (of the original 30), 17 for the mated-twice treatment (of the 18 that mated-twice), and 16 for the delayed-mating treatment (of the original 25). When the offspring laid in these vials emerged as adults, they were scored for their phenotypic markers. On average, there were 52 ± 27.2 (SD) offspring scored per vial.

In experiment 1 the mated-twice treatment comprised females that we observed multiply mating when presented with additional males. This approach could bias the effect of mating on recombination if females with high recombination are more likely to multiply mate. To avoid this problem, in experiment 2 we imposed a mating treatment that varied female exposure to males, similar to studies of the effects of multiple mating on female survival (Fowler and Partridge 1989; Chapman et al. 1995; Priest 2006). For both marker intervals (Pr–Dr, Bc–Kr), 192 virgin

females that were heterozygous for the markers were collected at hour 0 from vials that had been cleared 4 h before. From 48 to 72 h (female age two days), all of the females were exposed to three wild-type virgin males. At 72 h the males were discarded and the females were randomly assigned one of three treatments: high, medium, or low mating (Fig. 1B). The high-mating mothers were exposed to three new virgin males every day for six additional days. The males were discarded after each 24-h exposure. Medium-mating mothers were additionally exposed to three new virgin males from hour 120 to 144 (female age five days). Low-mating females did not receive any additional matings. For each marker interval the mothers were randomly assigned positions in one of eight trays, which were subsequently treated as blocks in the statistical analysis. The eggs that were deposited in the bottom of the vials were collected from each mother from hour 168 to 216 (female age seven to eight days). The mothers were discarded after their eggs were collected. The eggs were counted in each vial to measure egg-to-adult viability. After the adults had emerged the vials were flash frozen and the phenotypes of all of the offspring were scored. For the Pr–Dr interval 95 vials were scored (32 vials/mating treatment), with an average of 51 $\pm$ 17.2 (SD) offspring scored per vial. For the Bc–Kr interval 126 vials were scored (42 vials/mating treatment), with an average of 64 $\pm$ 19.6 (SD) offspring scored per vial.

The mating trials that we had conducted previous to this study indicated that low and medium mating females usually mate within the first 3 h of the 24-h male exposure and do not subsequently mate. This means that the females in experiment 2 would have been prevented from additional mating for 117, 45, or zero hours (low, medium, and high mating, respectively) before the start of the 48-h egg collection.

STATISTICAL ANALYSIS

The effect of the mating treatments on recombination rate was analyzed with log-linear analysis, using Proc GENMOD assuming a binomial distribution and a logit link in SAS (SAS 2003). For experiment 1, the effect of treatment was first evaluated across the entire experiment. Then, contrasts were used to test a priori hypotheses. Specifically, the effect of maternal age on recombination was evaluated by comparing recombination of once-mated females aged three to seven days with those for delayed-mating females aged 21–26 days. The effect of additional mating on recombination was tested by comparing the recombination of once-mated females aged 21–26 days (eggs collected late) with those for twice-mated females aged 21–26 days. Finally, the effect of mating history on recombination was determined by comparing the recombination of delayed-mating females aged 21–26 days with those for twice-mated females aged 21–26 days (see Fig. 1). Roughly 25% of the treatment animals died before, or failed to lay viable eggs during, the 21- to 26-day interval; we tested for survival bias between the mating treatments using a chi-squared test.

For experiment 2, mating treatment, marker interval, and the block nested within marker interval were tested. Following a finding of significant mating effects, contrasts were used to test all possible comparisons of the three mating treatments. To visually compare differences in recombination rate between intervals of different length, we standardized recombination frequency by dividing recombination frequency by the length of the marker interval.

Results

In experiment 1 the treatments introduced a significant variation in recombination rate ($\chi^2 = 42.75$, 4 df, $P < 0.0001$; Fig. 2). Maternal age had a large effect on recombination. Recombination rate was 47% higher for females that were first mated at an older age (delayed-mating) than for females that were first mated at a young age (once mated (eggs collected early)) ($\chi^2 = 30.75$, 1 df, $P < 0.0001$; Fig. 2). Additional mating also had a significant influence on the recombination rate. Twice-mated females had 18% greater recombination rate than once-mated females (the eggs were collected at the same late age) ($\chi^2 = 5.95$, 1 df, $P = 0.015$; Fig. 2). The effect of mating on recombination did not depend on a female's previous mating history. The recombination rate of females first mated at a young age and also mated at an older age (twice mated) was not significantly different than females that were first mated at an old age (delayed-mating) ($\chi^2 = 0.01$, 1 df,

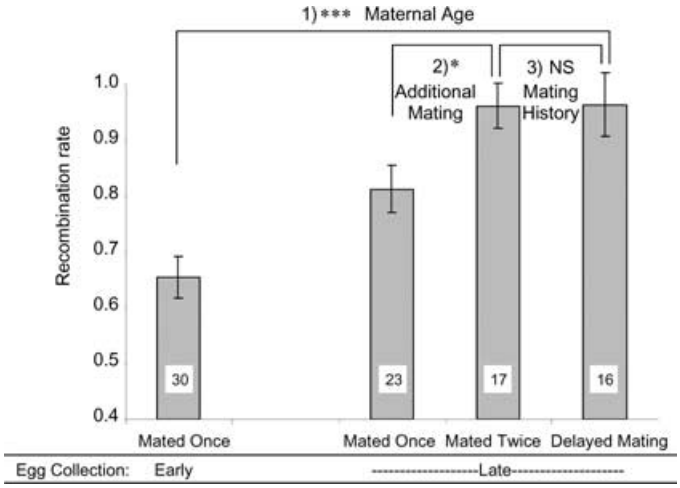

Figure 2. The average recombination rate, with standard error, for the four treatments of experiment 1. The recombination rate was standardized by dividing the proportion of recombinants by the length of the cl–cn marker interval. Internal numbers are numbers of mated mothers. Three linear contrasts based on log-linear analysis were performed to test for effects of (1) maternal age, (2) additional mating, and (3) mating history on recombination. Significance is indicated as ns $P > .05$, $^{*}P < 0.05$, $^{***}P < 0.001$.

Table 1. Log-linear analysis of experiment 2 testing whether mating frequency influenced recombination rate. Test included two marker intervals and maternal and offspring flies were grouped into blocks. Effects with $P < 0.05$ are in bold.

Source	df	χ^2	$P<\chi^2$
Mating treatment	2	9.17	**0.0102**
Marker interval	1	310.22	**<0.0001**
Block (interval)	14	24.50	**0.0399**
Mating*interval	2	2.09	0.3514

$P = 0.908$; Fig. 2). There was no evidence that differences in survival between the treatments biased the results of experiment 1 ($\chi^2 = 2.102$, 3 df, $P = 0.55$).

In experiment 2, the mating treatment also influenced the recombination rate (Table 1). Overall, females with recent and daily exposure to males had 12% higher recombination than females with only two 24-h exposures to males, and 20% higher recombination than females who had mated-once and were subsequently withheld from additional male exposure (Fig. 3). There were significant differences in the recombination rate between high- and low-mating females ($\chi^2 = 7.43$, 1 df, $P = 0.006$), high- and medium-mating females ($\chi^2 = 6.31$, 1 df, $P = 0.012$), but not medium- and low-mating females ($\chi^2 = 0.02$, 1 df, $P = 0.883$). The significant effect of marker interval was expected because the map distance (and thus predicted recombination frequency) of the Bc–Kr interval was three times larger than the Pr–Dr interval (Table 1). The marker intervals responded similarly to the mating treatment (no mating treatment x marker interval interaction) (Table 1). There was no effect of mating treatment on egg production ($F_{2,219} = 1.256$, $P = 0.29$) or egg-to-adult viability ($F_{2,219} = 0.017$, $P = 0.98$). There was also no correlation between egg production and recombination rate ($r = -0.104$, $N = 221$, $P = 0.13$).

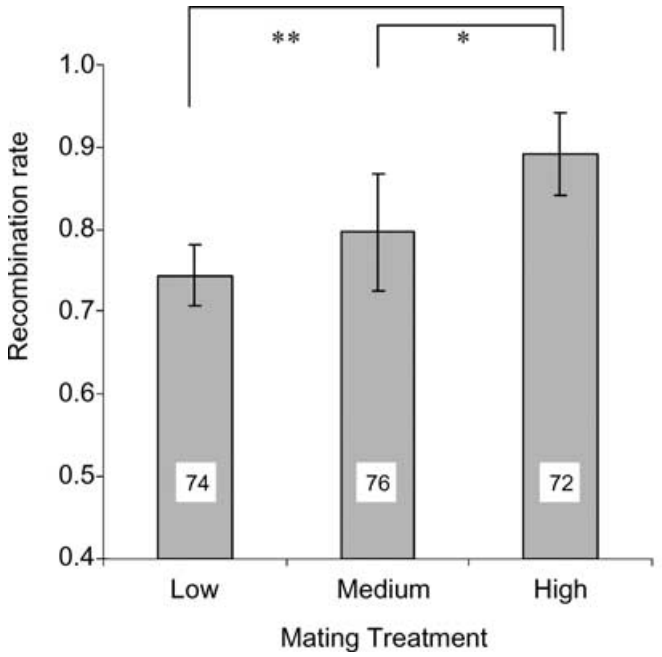

Figure 3. The average recombination rate, with standard error, for the high-, medium-, and low-mating treatments from experiment 2. The recombination rate was standardized by dividing the proportion of recombinants by the length of the marker interval. Internal numbers are sample sizes. Differences between treatments were evaluated using linear contrasts based on log-linear analysis (*$P < 0.05$, **$P < 0.01$).

Discussion

The recombination rate was influenced by the mating and age treatments. There are several potential causes for the observed variation in recombination rate, including the total number of matings, how recently the females had mated, mating frequency, female age, the current rate of egg production, and the prior amount of eggs produced. Though we could not distinguish among all of the potential causes, the results indicate that the age of females and how recently they mated influenced the recombination rate.

In experiment 1, females that mated-twice had 18% higher recombination rate than females that mated-once at an early age. This indicates that additional mating events boost the recombination rate and, at first sight, appears to suggest that females that have a greater total number of matings have a greater recombination rate; however, this is not the case. The mating history effect shows that females that mated-twice had an equivalent recombination rate to females that mated-once at a late age when recombination was assessed immediately after the late-age matings. There are two explanations for this finding. One hypothesis is that late-age copulations increase recombination rate. The other hypothesis is that each bout of mating temporarily increases recombination rate. Experiment 2 supports the latter hypothesis. We measured the recombination rate of females at young ages that had been prevented from additional mating for 117, 45, or zero hours (low, medium, and high mating, respectively) before the start of egg collection. We found that the females that were allowed to mate before and throughout the 48-h egg collection interval had the highest recombination rate; females that were allowed to mate around 45 h before the start of egg collection had a lower recombination rate and females that were prevented from additional bouts of mating before egg collection had the lowest recombination rate. Thus, females that had been exposed to males most recently had a higher recombination rate. These results indicate that each bout of mating temporarily increases recombination. It also suggests that it is possible to observe higher recombination rates in infrequent-mating females if they mated more recently than frequently mating females.

In experiment 1 we assessed the effects of maternal age on the recombination rate while accounting for the effects of mating by comparing the recombination rate of females immediately after their first mating at a young age and immediately after their first mating at an older age. The recombination rate within the marker interval was greater for older mothers than younger ones. This maternal age effect on recombination is similar to previous work (Bridges 1929; Neel 1941; Redfield 1966), and may have adaptive consequences. For example, because older mothers could be more likely to be diseased than younger mothers, there might be selection on increased recombination with maternal age to offset the higher risk of offspring infection. Maternal age effects on recombination might also alter the response to age-specific selection, which, for example, could affect the rate of the evolution of aging. The maternal age effect was not likely to have been biased by cohort heterogeneity (e.g., Vaupel and Yashin 1985) because in the mated-once treatment the seven females that died before the late-age egg collection had an equivalent recombination rate to the females that survived.

The effects of recent mating events on the recombination rate might be confounded by birth-order effects on recombination or by the effects of mating on egg production. For example, Redfield's (1966) study of fruit fly maternal age effects found that the recombination rate progressed with time in a consistent pattern, from being initially high, to low, to high again after 10 days. But the start of progression in the recombination rate with time was not determined by the age of the females; instead, it was determined by the age at which the females first mated. Because females begin laying eggs in copious quantities when they first mate, it seems plausible that the recombination rate of a particular egg is determined by how many eggs had been laid before that egg. Our results might also have been influenced by birth-order effects. In experiment 2 high-mating females could have had a higher recombination rate if they had produced more eggs than low-mating females, which could have shifted their eggs more rapidly to a higher recombination rate than low-mating females through birth-order effects. Redfield (1966) also found an inverse relationship between egg production and recombination rate (greater egg production, lower recombination rate). This suggests that ovule production could determine recombination rate if mating bouts boost ovule production and if recombination rate covaries with ovule production. We did not design the experiment to address this idea, thus we cannot eliminate birth-order effects or egg production as potential sources of variation in recombination rate. However, several previous studies have found no effects of mating and mating frequency on egg production at early ages in the Dahomey line used in experiment 2 (Fowler and Partridge 1989; Friberg and Arnqvist 2003; Brown et al. 2004; Priest 2006). In addition, mating did not affect egg production during the collection interval and there was no correlation between egg production and recombination rate in experiment 2.

Another explanation is that female recombination might be directly influenced by compounds produced in male accessory glands (Acps), which are delivered to females during mating and can reduce female survival (Chapman et al. 1995). If Acps directly interact with oocytes before recombination takes place, perhaps by interfering with DNA repair processes or the formation of recombination nodules where meiotic exchange takes place, then male-derived compounds, not changes in female physiology, might affect recombination. However, this possibility is also unlikely. Females appear to mediate physiological responses after mating and exposure to Acps (Wolfner 2002; Ravi Ram et al. 2005). For example, exposure to Acps and the act of mating stimulate the expression of over 1000 genes within the female genome (McGraw et al. 2004). Mating and exposure to Acps also have stressful effects on females (Chapman et al. 1995; Wolfner 2002), which could potentially stimulate stress-induced recombination processes (Parsons 1988; Hoffman and Parsons 1997; Badyaev 2005).

The physiological mechanisms that give rise to mating-induced recombination need further investigation. In all eukaryotes, recombination nodules form in premeiotic oocytes, but the amount of crossing over is not actually resolved until chromosome segregation at metaphase I (Page and Hawley 2003). In *D. melanogaster*, approximately 10 days elapse from the start of oogenesis to egg maturation, with oocyte stages 1 through fully mature stage 14 eggs occurring in the final 79 h of development (Koch and King 1966; David and Merle 1968). In experiment 2, we found that the females that did not mate for 45–93 h before eggs were laid (medium mating) had a greater recombination rate than females who had not mated for 117–144 h (low mating) and a lower recombination rate than females who were held in constant exposure to males (high mating). This means that it is possible that oocyte stages 1 through 14 are potentially sensitive to the effects of mating on recombination. This range falls intermediate to the six- to seven-day time lag between heat stress and altered recombination rate (Grell 1971; Day and Grell 1976) and the one-day time lag between cold treatment and altered rate of nondisjunction (Tokunaga 1970), and is similar in timing to the effects of mating on egg production (Kalb et al. 1993) and egg viability (David 1963).

Recombination is evolutionarily important because it breaks up the linkage groups and generates novel genotypes (Eshel and Feldman 1970). Though genetic makeup can affect the speed and direction of how recombination evolves (Maynard Smith 1978; Barton 1995; Charlesworth and Barton 1996; Feldman et al. 1997; Peters and Lively 2000), in traditional deterministic models there is little variation in the recombination rate (Charlesworth 1993;

Kondrashov 1993; Barton 1995). In contrast to these expectations, we found that the recombination rate is surprisingly plastic to recent bouts of mating.

This finding has two evolutionary implications. First, it might broaden the conditions for the maintenance of sex in a manner similar to environment-induced recombination (Agrawal et al. 2005). Because of the problem of recombination load (Charlesworth and Barton 1996), the evolution of recombination is fairly restricted; however, recent models show that the conditions for the evolution of recombination are broad if recombination rate is plastic to changes in the environment (Hadany and Beker 2003; Agrawal et al. 2005). Mating and recombination rates might be linked for this purpose in fruit flies. Fruit flies adjust their mating rates according to the population conditions, and these fluctuate with available resources (Prout and McChesney 1985). Mating frequency is higher in high-resource conditions than in resource-limited conditions (Turner and Anderson 1983; Chapman and Partridge 1996). If recombination is beneficial in high-resource conditions, then mating-induced recombination would broaden the conditions for the maintenance of sex.

Second, mating-induced recombination may have adaptive significance for the evolution of sexual behavior. Females from sexually reproducing species typically mate with multiple males (Arnqvist and Nilsson 2000). In many taxa, multiple mating has been shown to decrease female survival and total fecundity (Fowler and Partridge 1989; Gems and Riddle 1996; Crudgington and Siva-Jothy 2000; Arnqvist and Nilsson 2000; Priest 2006). One of the arguments for why females mate with multiple males, despite substantial fitness costs, is that receiving sperm from multiple males can increase the genetic variability of offspring (Jennions and Petrie 2000; Kokko et al. 2003; Pizzari and Snook 2003). However, this type of genetic benefit of multiple mating is expected to generate only small improvements in fitness (Yasui 2001). Another possibility is that multiple mating might affect offspring fitness through parental effects (Qvarnstrom and Price 2001; Kozielska et al. 2004). Recent work indicates that bouts of additional mating can induce parental effects, which increase the fitness of daughters (Priest 2006). Here we show that additional bouts of mating can cause a pulsed increase in the genetic variability of the offspring through mating-induced recombination. These results indicate that there are both genetic and nongenetic indirect benefits of frequent mating for females, which could broaden the conditions for the evolution of multiple mating. Thus, mating-induced recombination might help overcome the genetic costs of sex and the fitness costs of frequent mating.

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