

Putative Condition-Dependent Viability Selection in Wild-Type Stocks of *Drosophila pseudoobscura*

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Abstract

Meiotic recombination rates vary in response to intrinsic and extrinsic factors. Recently, heat stress has been shown to reveal plasticity in recombination rates in *Drosophila pseudoobscura*. Here, a combination of molecular genotyping and X-linked recessive phenotypic markers were used to investigate differences in recombination rates due to heat stress. In addition, haplotypes from the genetic crosses were compared to test if they deviated from equal proportions, which would indicate viability selection. To avoid this potential bias, SNP genotyping markers overlapping the regions assayed with mutant markers were used to further investigate recombination rate. Interestingly, skews in haplotype frequency were consistent with the fixation of alleles in the wild-type stocks used that are unfit at high temperature. Evidence of viability selection due to heat stress in the wild-type haplotypes was most apparent on days 7–9 when more mutant non-crossover haplotypes were recovered in comparison to wild type ($p < 0.0001$). Recombination analysis using SNP markers showed days 9–10 as significantly different due to heat stress in 2 pairs of consecutive SNP markers ($p =$

0.018; $p = 0.015$), suggesting that during this time period the recombination rate is most sensitive to heat stress. This peak timing for recombination plasticity is consistent with *Drosophila melanogaster* based on a comparison of similarly timed key meiotic events, enabling future mechanistic work of temperature stress on recombination rate.

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Introduction

Meiosis is fundamental for sexually reproducing organisms to generate haploid gametes. This process helps to maintain the correct number of chromosomes in the next generation, critical for zygote viability. Additionally, crossing over during meiosis creates novel genetic variation by recombining parental haplotypes, which can have important consequences for adaptation of species [Charlesworth and Barton, 1996; Page and Hawley, 2003].

Early studies in *Drosophila melanogaster* have shown that crossover rates vary as a result of various factors including maternal age, starvation, as well as external humidity and temperature [Plough, 1917, 1921; Bridges, 1927; Kohl and Singh, 2018; Singh, 2019]. In more recent studies, it has been shown that infection also alters re-

combination rate frequencies [Singh et al., 2015; Singh, 2019]. Over the last century, other model systems have replicated these results [reviewed in Parsons, 1988; Agrawal et al., 2005; Bomblies et al., 2015; Modliszewski and Copenhaver, 2017]. For example, results from more recent studies indicate that desiccation is a recombinogenic factor and that desiccation-induced changes in both recombination rate and crossover interference are fitness-dependent, with a tendency of less fit individuals producing more variable progeny. Such dependence may play an important role in the regulation of genetic variation in populations experiencing environmental challenges [Aggarwal et al., 2019].

While these factors have consequences on events throughout meiosis such as in synaptonemal complex and double-strand break formation, early meiosis appears to be most sensitive to perturbation by a number of factors leading to apoptosis in these stages [reviewed in Stevison et al., 2017; Singh, 2019]. Experimental evidence points to temperature-sensitive, pre-meiotic interphase as the stage when plasticity in recombination rate is the highest. This coincides with the relationship between DNA replication at S-phase and meiotic recombination [Grell, 1973, 1978b].

While there has been a century of work on recombination rate plasticity in *D. melanogaster*, there have been no efforts to document this phenomenon in other *Drosophila* species. The *Drosophila* genus diversified over 50 million years ago and comprises over 2,000 extant species [Hales et al., 2015]. Moreover, Parsons [1988] argued that *Drosophila* species can serve as indicators of global climate change due to their environmental sensitivity. However, one concern with focusing on *D. melanogaster* in the study of how environmental stress impacts recombination is that as a cosmopolitan species, it may not have the same environmental sensitivity as other species within the *Drosophila* species group. Our team has recently worked to expand research on this ubiquitous phenomenon into *Drosophila pseudoobscura* [Stevison et al., 2017].

D. pseudoobscura is native to western North America and a small region in Bogota, Colombia. It is therefore alpine over parts of its range, which means it has the potential to be more sensitive to environmental changes [Kuntz and Eisen, 2014]. This species of *Drosophila*, which is approximately 30 million years diverged from the classic model, *D. melanogaster* [Throckmorton, 1975], was the second *Drosophila* species to have its genome completely sequenced and is traditionally studied for inversion polymorphisms, which makes it a good model for

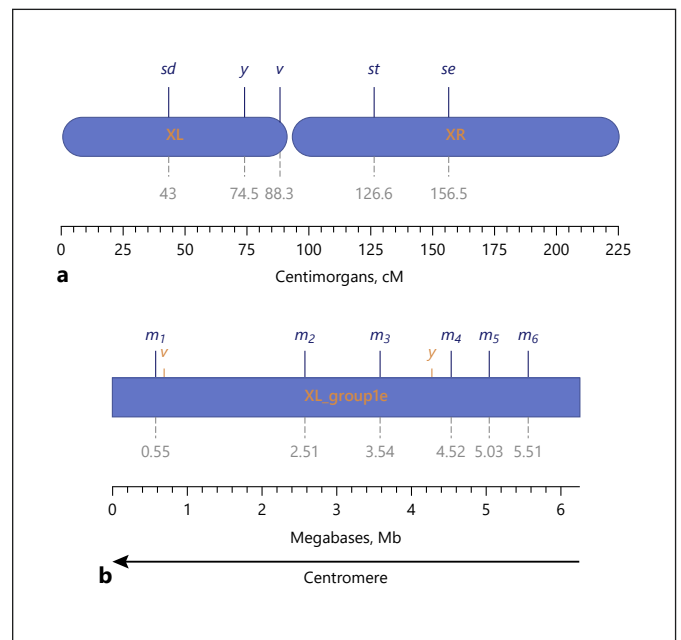
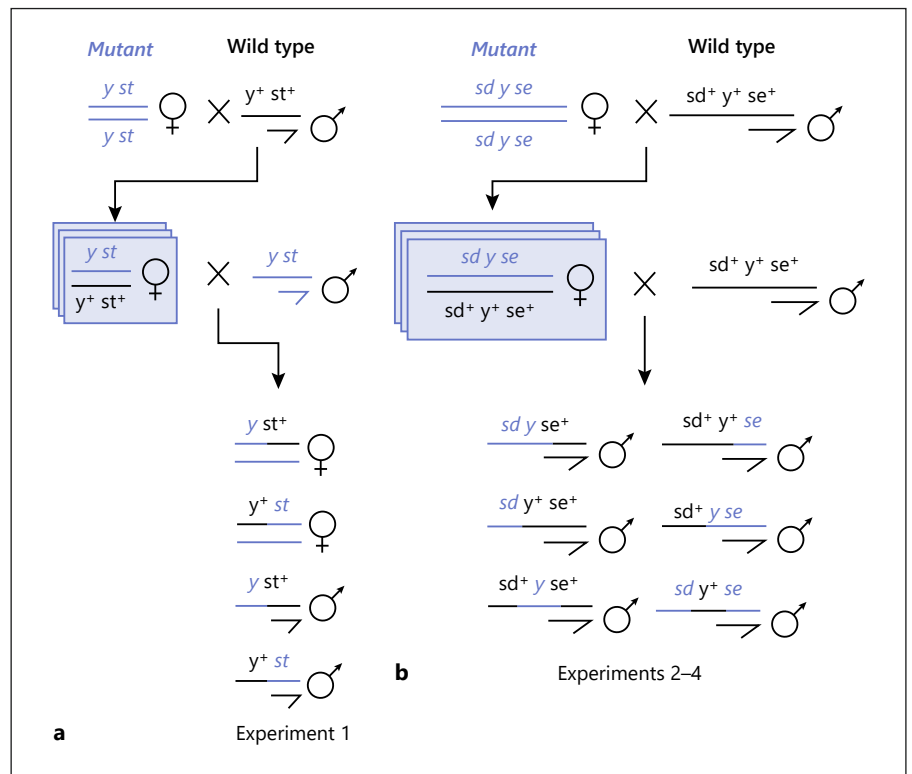


Fig. 1. Summary of experimental design and results to measure the impact of temperature and age on recombination frequency. **a** Genetic map of the X chromosome with location of mutant X-linked markers *scalloped* (*sd*), *yellow* (*y*), *scarlet* (*st*), and *sepia* (*se*) used to measure viability and recombination in Experiments 1–4. **b** Physical locations of SNP genotyping markers along 12.5 Mb scaffold “XL_group1e” located on the left arm of the X chromosome (XL). This scaffold (shown in reverse orientation) covers 62% of XL (only half shown here), including the mutant markers *vermillion* (*v*) and *yellow* (*y*).

recombination studies [Hales et al., 2015]. Additionally, *D. pseudoobscura* females exhibit synchronization of oogenesis across egg chambers [Donald and Lamy, 1938], which is key to studying the timing of events in meiosis because time is an indicator of progression through oocyte development. More recently, there has been a boost of interest in studying recombination rates in this species [Kulathinal et al., 2008, 2009; Stevison and Noor, 2010; McGaugh et al., 2012; Samuk et al., 2020].

Our lab recently reported a preliminary analysis of recombination rate plasticity due to heat treatment during development in *D. pseudoobscura* [Stevison et al., 2017]. In that study, significant plasticity was found in 8 regions across the 2nd chromosome, with 5/8 regions showing higher recombination in the high temperature treatment (see Table S1 in Stevison et al. [2017]). These results parallel both classic and recent work done in *D. melanogaster* [Grell, 1966, 1973, 1984; Singh et al., 2015; Ritz et al., 2017; Kohl and Singh, 2018].

Fig. 2. Crossing scheme for experiments using mutant phenotypes. Females homozygous for mutant markers of 2 stocks were used to cross to wild-type flies (indicated by plus sign). This F₁ cross was the unit of replication, as indicated by the stacked boxes, and the resulting female progeny experienced the treatments as indicated in Table 1. The ID of these crosses were tracked in the resulting backcrosses. **a** In Experiment 1, the *y st* mutant stock and the MV2-25 wild-type stock were used. **b** For Experiments 2–4, the triple mutant stock *sd y se* and the SCI_12.2 wild-type stock were used. Virgin F₁ females were collected and stored in a common control temperature prior to the backcrosses. Based on initial screening of male backcross progeny, the marker *ct* was removed from consideration as it gave unreliable results due to incomplete penetrance. Male backcross progeny were screened for recombination analysis (Eq. 2) and female progeny were included for fecundity analysis (Eq. 1).



Here, this work was continued to establish *D. pseudoobscura* as a model for studying recombination rate plasticity. First, a series of experiments was conducted with the goal of pinpointing the timing of peak differences in recombination rate between control and temperature stress crosses. Temperature was used as treatment throughout development similar to the work of Plough and others [Plough, 1917, 1921; Stevison et al., 2017], as well as maternal age. Phenotypic mutants were used, and the experimental parameters were adjusted with each successive experiment, altering treatment between temperature and age, duration of progeny collection, progeny transfer frequencies, and sample sizes. Although the cross design primarily backcrossed to wild-type flies to mitigate potential viability effects of the mutant markers, a thorough investigation into the haplotype frequencies from the mutant marker crosses was conducted to test for segregation bias. This analysis revealed these crosses to have significant deviations from the expectation of equal proportions based on Mendel's first law. Interestingly, these results seemed to change between treatment and control as well as time points, suggesting condition-dependent variability in viability of the wild-type alleles. Finally, SNP genotyping markers were

used to confirm the recombination results from the phenotypic mutants due to their evidence for viability selection. Combining strategies used in earlier studies, the work presented here provides important information for future mechanistic work to understand recombination rate plasticity and enable it to be studied in more depth in *D. pseudoobscura*.

Materials and Methods

Stocks

Genetic crosses using mutant markers were conducted using 2 X-linked recessive mutant *D. pseudoobscura* stocks. First, a double mutant stock was produced by crossing 2 lines obtained from the UC San Diego stock center (which has relocated to Cornell University): *yellow* (*y*; 1-74.5) found on the first chromosome (or chromosome X) at genetic map position 74.5 (stock 14044–0121.09, *Dpse*[y[1]) and *scarlet* (*st*) (stock 14011–0121.06, *Dpse*[v[1])). Although there is not a precise map location for *scarlet* in the literature in *D. pseudoobscura*, it is consistently 30 cM away from *sepia*. This places it roughly 52 cM away from *yellow*, compatible with our observed recombination rates [Beers, 1937]. Mutations of the *scarlet* gene induce a bright red-eye phenotype [Beers, 1937], and mutations within the *yellow* gene induce a yellow-hued body and wings [Sturtevant and Tan, 1937]. Second, a triple mutant stock (courtesy of Nitin Phadnis) had 3 mutations: *yellow* (*y*; 1-74.5),

Table 1. Summary of experimental design and results to measure the impact of temperature and age on recombination frequency

	Treatments ^a	Transfer frequency/ duration	Number of replicate crosses	Median number of crosses per replicate	Sample size ^b	Fecundity ^c	Recombination ^d
Experiment 1 ^e	20°C/25°C	48H/6D; 72H/7–15D	8/9	10/10	5,187/4,568	0.24	0.05
Experiment 2 ^f	21°C/26°C	72H/12D	5/5	5/5	4,140/2,071	5.54E–11	0.76 (<i>sd-y</i>), 0.91 (<i>y-se</i>)
Experiment 3 ^f	21°C/26°C	24H/6–10D	6/6	12/12	3,425/2,084	4.49E–04	0.09 (<i>sd-y</i>), 0.59 (<i>y-se</i>)
Experiment 4 ^f	7-day/35-day	72H/12D	6/6	8/8	6,219/4,508	5.3E–3	0.93 (<i>sd-y</i>), 0.92 (<i>y-se</i>)
SNP genotyping	18°C/23°C	48H/10D	4/4	4/4	677/611	n/a	0.29

For each experiment, a different set of temperatures or ages, transfer frequencies and duration as well as sample sizes were used. ^a Slashes in columns 2 through 5 indicate values split by treatment and control as indicated in the treatments column. ^b Sample size is based only on the number of individuals targeted for recombination frequency (e.g., only males were phenotyped for Experiments 2–4). ^c For fecundity, values are based on all progeny of both sexes for the duration of the experiment but not over the lifetime of each replicate female. *p* value from Eq. 1 for treatment on fecundity. Significant values indicated in bold. ^d *p* value from Eq. 2 for treatment on recombination rate. Full Anova tables for both analyses and post hoc tests are in online supplementary Tables 3–8 (significant values indicated in bold). ^e Crossing scheme matches Figure 2a. ^f Crossing scheme matches Figure 2b. H, hours; D, days.

scalloped (*sd*; 1–43.0), and *sepia* (*se*; 1–156.5) [Phadnis, 2011]. Mutations of the *scalloped* gene induce changes to the wing phenotype in scallop shape, whereas mutations in the *sepia* gene result in brown eyes [Crew and Lamy, 1935]. Genetic locations of all mutant markers are shown in Figure 1a. A fourth mutant in the triple mutant stock (*cut*, *ct*; 1–22.5) produced inconsistent results likely due to a variation in penetrance of the mutation [Dworkin et al., 2009]. Therefore, the *ct* marker was excluded from the remainder of the analysis.

Three wild-type *D. pseudoobscura* stocks were also used for genetic crosses. First, MV2–25 was used in crosses to the double mutant stock since it represents the reference genome strain [Richards et al., 2005], and both are in an Arrowhead 3rd chromosome background. Second, to match the 3rd chromosome inversion arrangement of the multiple marker line, a second stock bearing the arrangement called “Treeline” was obtained from the National *Drosophila* species Stock Center at Cornell University (stock 14011–0121.265, Dpse/wild-type “TL”, SCI_12.2). This strain is also fully sequenced (NCBI Accession: SRX204785). Finally, AFC–57 [see Ritz et al., 2017] was used for indel genotyping because it was a readily available wild-type strain at the time.

Husbandry and Cross Design

All stocks were maintained at 21°C with a 12-h light-dark cycle in an incubator. Flies were reared on standard cornmeal-sugar-yeast-agar media in polypropylene vials.

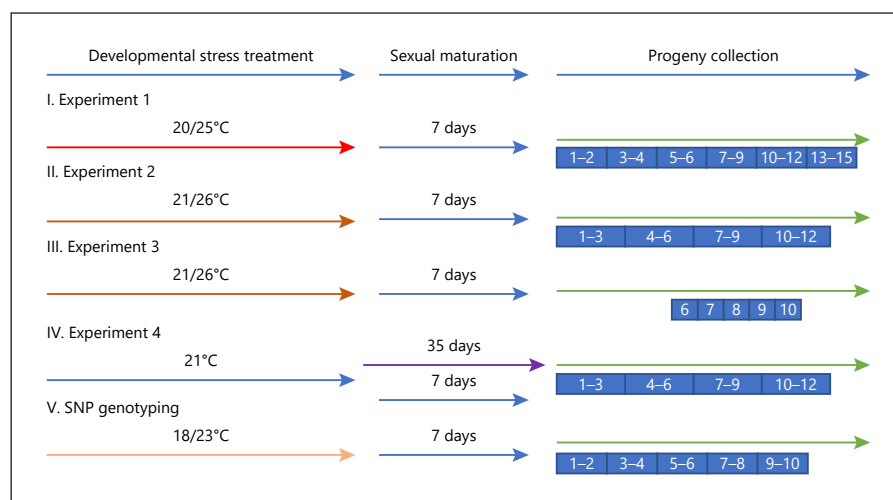
For indel genotyping, all crosses were performed at 20°C in glass vials containing 6 mL of corn syrup food. Virgin mutant female flies (5–7 days old) were crossed with male AFC–57. Virgin F₁ females (5–7 days old) were collected and crossed with mutant male flies (Fig. 2a). Resulting backcross progeny were phenotyped. Cross design for the SNP genotyping markers was described elsewhere [Stevison et al., 2017].

For genetic crosses, double and triple homozygous recessive mutant stock virgins were collected and aged 7 days to full sexual maturity. These flies were crossed to wild-type, age-matched males

in control conditions to produce heterozygous F₁ progeny (Table 1). To match the genetic background on the 3rd chromosome, wild-type flies crossed to the double mutants were in the arrowhead orientation (MV2–25), whereas for the triple mutants, they were in the treeline orientation (SCI_12.2). Virgin heterozygous F₁ females were collected within 8 h of eclosion and stored at 21°C to maintain a common developmental timeline for treatment and control. There, they were aged to 7 days and backcrossed to wild-type males reared at 21°C. This cross design using wild-type males also provided a built-in “fail safe” because female progeny could not be homozygous for the recessive mutant markers, and thus any mutant females would be an indicator of contamination. However, for Experiment 1, the backcross was done to the mutant stock (see below). Crossing schemes are diagrammed in Figure 2 with details on each experimental design outlined in Table 1, Figure 3, and below. Before backcrosses, wild-type males were individually isolated 24 h prior to crosses to avoid crowding-induced courtship inhibition [Noor, 1997]. To backcross, a single wild-type male and single F₁ female were placed in a fresh food vial. To increase sample sizes, multiple backcrosses were conducted from each replicate F₁ cross using sibling female progeny.

To promote mating, a cotton ball was placed inside to restrict available space and the vial was placed under a 100-W CFL light for an hour. After crosses, vials were assigned to identical incubators with a 12-h light-dark cycle with the temperature varying according to Table 1 resulting in thermal stress throughout development. After 24 h, the cotton was removed and the wild-type males were discarded to prevent additional stress from male harassment [Priest et al., 2007]. The females continued to be transferred to a fresh food vial according to the transfer frequency of each experiment (Table 1; Fig. 3; online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000522585). Additionally, the vials where virgins were held prior to genetic crosses were kept for 14 days to ensure there were no larvae. If larvae were found, the cross was discarded.

Fig. 3. Experimental design. Visual schematic of the experimental design for the series of experiments described. These parameters are also summarized in Table 1. For heat stress, F₁ females experienced a developmental difference in rearing temperatures. For maternal age, females were either 7 days (control) or 35 days (treatment) at mating. For each experiment, mated F₁ females were transferred with varying duration and frequency to partition the eggs laid into separate vials. All progeny from each vial as indicated by the blue boxes were collected for no more than a 2-week period of time to avoid overlapping generations of progeny.



Experimental Design

A series of 4 experiments were conducted using double (Experiment 1) and triple (Experiments 2–4) mutant stocks (summarized in Fig. 3). First, experiments were set up to investigate the impact of heat stress. The cross design for the first experiment was altered from the pilots to maximize sample size. Specifically, backcrosses were conducted to the X-linked recessive mutant stock rather than the wild-type stock as in the pilot experiments, allowing for the inclusion of female progeny in recombination calculations. Additionally, transfers were selected based on the aggregation of pilot experiment 1 data to hone in on the earlier time points with 48 h transfers for the first 6 days and 72 h transfers for the remaining 9 days, for 15 days total.

Next, to validate the findings in Experiment 1 using the triple mutant stock, Experiment 2 closely matched Experiment 1 modifying the transfer frequency to 72 h for simplicity. Additionally, because there was no effect of temperature on fecundity in experiments at 25°C, the temperature treatment was increased to 26°C to increase the temperature stress. In Experiment 3, the 7–9-day post-mating time period was honed in with 24 h transfers. However, to maximize the sample sizes in the later time points, both the number of replicates and crosses were increased relative to Experiment 1 and 2. Additionally, the vials where females were held for the first 5 days were discarded to keep the total sample size manageable.

Finally, to investigate the impact of maternal age, a fourth experiment was conducted closely matching the transfer frequency and duration of Experiment 2 (online suppl. Fig. 5). The heterozygous F₁ females were aged to 7 days (control) and 35 days (maternal age treatment) and backcrossed to wild-type males. The F₁ crosses were staggered so that the 7-day-old control females were backcrossed at the same time as the 35-day-old maternal age “treatment” flies. As shown in online supplementary Figure 5b, the F₁ females for the maternal age treatment were transferred into new vials every 7 days until they were 35 days old. When the maternal age treatment females were 35 days old and the control females were 7 days old, they were backcrossed to wild-type males.

The SNP genotyping experimental design was described in Stevenson et al. [2017] and is summarized in Table 1 and Figure 3. The SNP marker design is described below.

Phenotypic Scoring of Mutant Progeny

Resultant progeny were screened for presence or absence of the mutant markers (Table 1). Except for Experiment 1, only male progeny were scored and if any female progeny were found to be mutant, the entire vial was discarded and the data removed. Visual scoring of mutant markers recorded each of the mutant traits independently in a single-blind manner. For Experiments 2–4, mutant scoring was delayed at least 5 days for the *sepia* eye color to become more pronounced. Phenotyping ended 2 weeks after eclosion started to prevent the next generation from being included in the data. Data were entered in triplicate and compared until 100% concordant.

Sequenom SNP Genotyping

As part of a preliminary characterization of plasticity in *D. pseudoobscura*, Sequenom SNP genotyping markers were designed to genotype crosses between FS14 and FS16 wild-type flies (methods previously described in Stevenson et al. [2017]). Previously described results captured chromosome 2. In addition, for this study, 6 additional SNP markers were designed on the left arm of the X chromosome (chrXL) to span the region containing the mutant markers *yellow* and *vermillion* (Fig. 1b). Together, the 5 intervals span 5 Mb of the XL and are located on scaffold chrXL_group1e of the *D. pseudoobscura* reference genome.

Mutant Phenotype Segregation Analysis

Statistical analysis was performed using R v4.0.1 [R Core Team, 2020]. For each experiment, haplotypes were grouped within crossover classes in order to investigate viability differences. The data from the backcross progeny were summed over up to 8 different types of haplotypes (Table 2). Additionally, the progeny were split based on both time point and treatment in Table 3. Because of the expectation of equal segregation of haplotypes during meiosis, a binomial test was performed in order to test for statistical deviations from 50:50 for each haplotype combination. Significant skews from expectation are indicated in bold with asterisks used to denote statistical significance (Tables 2, 3). Additionally, the deviation from 50:50 was calculated across replicates for each crossover class and treatment (Fig. 4).

Table 2. Haplotype frequencies for Experiments 1–4

Experiment 1									
CO class	NCO		SCO						
Haplotype	<i>y +</i>	<i>+ st</i>	<i>++</i>	<i>y st</i>					
Offspring, <i>n</i>	2,388	2,220	2,674	2,473					
Bias ratio	0.93		0.92						
Experiment 2									
CO class	NCO		SCO1		SCO2		DCO		
Haplotype	<i>sd y se</i>	<i>+++</i>	<i>+ y se</i>	<i>sd ++</i>	<i>sd y +</i>	<i>++ se</i>	<i>+ y +</i>	<i>sd + se</i>	
Offspring, <i>n</i>	2,191	4,177	1,585	1,026	1,842	3,305	1,238	665	
Bias ratio	0.52**		0.65**		0.56**		0.54**		
Experiment 3									
CO class	NCO		SCO1		SCO2		DCO		
Haplotype	<i>sd y se</i>	<i>+++</i>	<i>+ y se</i>	<i>sd ++</i>	<i>sd y +</i>	<i>++ se</i>	<i>+ y +</i>	<i>sd + se</i>	
Offspring, <i>n</i>	1,697	4,197	1,309	863	1,653	2,877	949	450	
Bias ratio	0.40***		0.66*		0.57*		0.47**		
Experiment 4									
CO class	NCO		SCO1		SCO2		DCO		
Haplotype	<i>sd y se</i>	<i>+++</i>	<i>+ y se</i>	<i>sd ++</i>	<i>sd y +</i>	<i>++ se</i>	<i>+ y +</i>	<i>sd + se</i>	
Offspring, <i>n</i>	2,721	3,951	1,610	1,392	2,543	2,554	1,408	716	
Bias ratio	0.69*		0.86*		1.00		0.51*		

Haplotype frequencies for each crossover class from each experiment using phenotypic mutant markers were analyzed to investigate possible segregation bias due to potential viability effects of visual markers for measuring recombination rate. A binomial test was performed to test for unequal proportions for each crossover class pair (bold values indicate significance; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Bias ratio was calculated as the minimum divided by the maximum to keep values below 1 for better comparison. Additional breakdown of haplotypes by time point and treatment are shown in online supplementary Table 9. Variation of bias ratio across F_1 replicates and treatment are shown in Figure 4. NCO, non-crossover; SCO, single crossover; DCO, double crossover.

Statistical Analysis of Fecundity

Additionally, fecundity was tracked to measure the impact of stress due to temperature treatment and was calculated by dividing the number of backcross progeny to the number of F_1 mothers. A quasi-Poisson regression analysis was conducted following a similar basic model equation:

$$F = V + D + T + D*T \quad (\text{Eq. 1})$$

“F” indicates the continuous response variable of total number of progeny, or fecundity, for each time point. “V” indicates the replicate vial ID and corresponds to F_1 crosses. “D” indicates the transfer period, or days post-mating, of the F_1 female. Finally, “T” indicates the temperature at which the F_1 female was reared. For each replicate cross, fecundity was summed over all crosses and divided by the number of crosses per replicate to get an average number of progeny per time point for each replicate. Additionally, a post hoc lsmeans contrast was conducted to compute the significance of

treatment versus control for each time point in each experiment (see online suppl. Tables 7 and 8).

Statistical Analysis of Recombination Frequency

Recombination rate frequencies were calculated for the chromosomal interval between each phenotypic marker (Fig. 1a). Recombination frequencies correlating mapping distance between linked alleles were calculated by dividing the number of recombinant flies for regions *y-st*, *sd-y*, or *y-se* to the total number of progeny.

Glmer function was used to generate a fitted model using logistic regression per interval with replicate vial IDs as random effects and all other parameters as fixed effects. For each interval within each experiment, a logistic regression analysis with a mixed model was conducted in R. The basic model equation was:

$$R = V + D + T + D*T \quad (\text{Eq. 2})$$

Table 3. Haplotype frequencies for each experiment are provided as in Table 2, but here broken down further by treatment and time point

Experiment 1							
Haplotypes	Treatment, °C	Days 1–2	Days 3–4	Days 5–6	Days 7–9	Days 10–12	Days 13–15
<i>y +</i>	20	374	318	206	138**	114*	102**
<i>+ st</i>	20	383	354	210	76**	78*	45
<i>+ +</i>	20	441	378	204	163*	152**	163**
<i>y st</i>	20	451	343	238	90	99**	67**
<i>y +</i>	25	401	372	72	164**	79	48**
<i>+ st</i>	25	440	428	64	68**	59	15**
<i>+ +</i>	25	414	464	47*	102	116*	30*
<i>y st</i>	25	461	475	85*	91	61*	12*
Experiment 2							
Haplotypes	Treatment, °C	Days 1–3	Days 4–6	Days 7–9	Days 10–12		
<i>sd y se</i>	21	212***	160***	145***	92*		
<i>+ + +</i>	21	309***	282***	36***	123*		
<i>+ y se</i>	21	149***	116	142***	76		
<i>sd + +</i>	21	96***	94	68***	66		
<i>sd y +</i>	21	185	154*	119***	95***		
<i>+ + se</i>	21	217	196*	204***	159***		
<i>+ y +</i>	21	129***	92	88*	75*		
<i>sd + se</i>	21	79***	74	61*	47*		
<i>sd y se</i>	26	119**	93	58***	43		
<i>+ + +</i>	26	174**	117	24***	49		
<i>+ y se</i>	26	73	65	53	37		
<i>sd + +</i>	26	73	47	50	24		
<i>sd y +</i>	26	96	75	66**	39**		
<i>+ + se</i>	26	117	94	102**	73**		
<i>+ y +</i>	26	60	48**	47	29		
<i>sd + se</i>	26	48	24**	33	21		
Experiment 3							
Haplotype	Treatment, °C	Day 6	Day 7	Day 8	Day 9	Day 10	
<i>sd y se</i>	21	125***	46**	84*	105	86*	
<i>+ + +</i>	21	234***	106**	190*	102	142*	
<i>+ y se</i>	21	109***	51**	74	64	54	
<i>sd + +</i>	21	67***	20**	63	53	63	
<i>sd y +</i>	21	143	61*	90*	100	98	
<i>+ + se</i>	21	179	93*	129*	117	118	
<i>+ y +</i>	21	71**	50**	74*	55	48	
<i>sd + se</i>	21	38**	14**	45*	34	30	
<i>sd y se</i>	26	78***	32**	64*	41	98	
<i>+ + +</i>	26	126***	75**	90*	32	128	
<i>+ y se</i>	26	37	35*	62**	35	48	
<i>sd + +</i>	26	32	18*	28**	37	50	
<i>sd y +</i>	26	52*	31*	51	39*	78	
<i>+ + se</i>	26	98*	65*	67	64*	83	
<i>+ y +</i>	26	34	31**	45	29	52**	
<i>sd + se</i>	26	30	9**	35	19	26**	

Table 3 (continued)

Experiment 4					
Haplotypes	Treatment	Days 1–3	Days 4–6	Days 7–9	Days 10–12
<i>sd y se</i>	7-day	427*	297*	215*	89
<i>+++</i>	7-day	543*	366*	171*	110
<i>+ y se</i>	7-day	217**	188	131	73
<i>sd ++</i>	7-day	283**	169	111	62
<i>sd y +</i>	7-day	408**	293	171	82
<i>++ se</i>	7-day	330**	251	201	91
<i>+ y +</i>	7-day	219*	187**	123**	55
<i>sd + se</i>	7-day	163*	74**	78**	41
<i>sd y se</i>	35-day	199**	231*	128	101*
<i>+++</i>	35-day	332**	314*	165	142*
<i>+ y se</i>	35-day	141	140	71	97*
<i>sd ++</i>	35-day	136	143	81	63*
<i>sd y +</i>	35-day	183	221	123	108*
<i>++ se</i>	35-day	197	198	140	152*
<i>+ y +</i>	35-day	155*	138**	70	77**
<i>sd + se</i>	35-day	116*	58**	52	36**

Binomial test was performed to test for the deviations between paired haplotype groups in the same experimental treatment/time point that should be in equal proportions (asterisks indicate significance; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Shown in bold/italic are NCO (non-crossover) class crossovers where there is a significant excess of mutant progeny as compared to wild type.

Here, all variables are the same as in Eq. 1, except the response variable, “R”, in this model is the binary response variable of whether an individual offspring was recombinant or not based on the pair of mutant phenotypes over the screened region, for each time point. Progeny from backcrosses of F₁ female siblings were summed per replicate cross per day and any replicate with fewer than 10 progeny were removed to avoid stochasticity in recombination rate estimates.

The results of both models are summarized in Table 1 and online supplementary Table 1, and the full model tables can be found in online supplementary Tables 3 and 4. Individual odds ratios were extracted for each time point using a post hoc means contrast between temperature and control to estimate biological relevance (Fig. 5; online suppl. Fig. 5, 6). For logistic regression, exponentiating the coefficients of GLMM generates the odds of crossover formation between experimental and control conditions. A post hoc lsmeans contrast was done to calculate significance for each time point between treatment and control within the overall model for each experiment (see online suppl. Tables 5, 6).

Molecular Genotyping to Investigate High Recombination Rate in Double Mutants

Molecular genotyping was used to confirm association between phenotypic mutants and their respective genes for the *yellow* and *vermilion* genes. For this analysis, 2 indel markers were designed based on the *D. pseudoobscura* assembly v3.1, each within 25 kb from the *vermilion* and *yellow* genes. Markers selected resulted in differing PCR product lengths between the mutant stocks and the wild-type AFC-57 stock (online suppl. Table 2). DNA was isolated [Gloor and Engels, 1992] from a minimum of 88 flies for each parent stock and backcross progeny for PCR amplification (Fig. 2a).

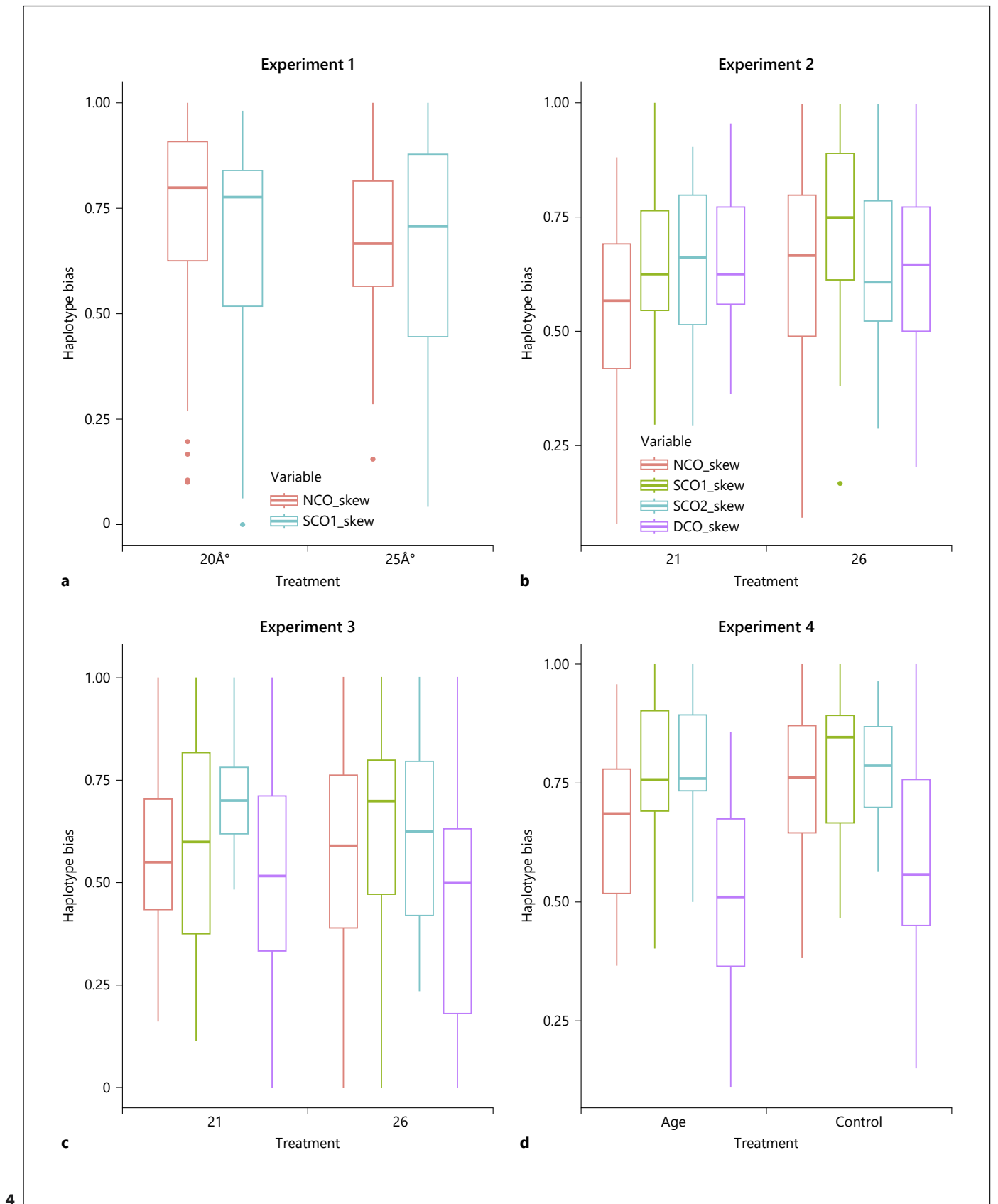
Length differences for markers were assayed via acrylamide gel. To confirm linkage between the *vermilion* and *yellow* genes and the red eye and yellow body phenotypes, backcross progeny of known phenotype were genotyped for the *vermilion*-linked and *yellow*-linked indel markers.

Survivorship Analysis

In order to determine the life span of *D. pseudoobscura*, F₁ females were generated using the same crossing scheme described for the recombination rate estimates. Eighteen replicate crosses of 10 mutant females with 5 wild-type males were conducted, and the F₁ female progeny were collected. Progeny were kept in vials with an average of 6.5 females (ranging from 1 to 13) based on when they were collected. To ensure the females had fresh food supply throughout the experiment, they were transferred to fresh food every 7 days. At each transfer, the number of females remaining in the vial was counted and recorded until no flies were left. For each replicate and time point, the percentage remaining as compared to the initial count was computed. The median across each time point was then computed to identify the time point at which less than 50% females remained. This analysis was used to justify the choice of age selected.

Results

A series of 4 experiments were conducted using double (Experiment 1) and triple (Experiments 2–4) mutant stocks (summarized in Fig. 3) to assay the impact of heat



(For legend see next page.)

stress and maternal age. While the main aims for these experiments were to assay the haplotype frequencies and compare the bias between different recombinant haplotypes, recombination rate variation was investigated as well. Differences in the timing of progeny collection (Fig. 3) were used to hone in on the most sensitive time point of recombination rate plasticity.

Experiments 1–4 used mutant markers which are known to have bias in haplotype frequency due to potential viability effects, therefore, we examined how this viability selection varied by treatment and time. We conducted a binomial test to determine if the differences in haplotype frequencies were significantly different from a 50:50 expectation (significant values bolded and asterisked in Tables 2, 3, and online suppl. Table 9). The 4 experiments showed condition-dependent variation in the overall skew from a 50:50 expectation (Fig. 4).

Double mutant cross reveals less overall viability selection than triple mutant crosses.

Experiments conducted using a double mutant stock (*y st*) crossed to the wild-type genome line MV2-25 (Fig. 2a; online suppl. Fig. 1) varied in transfer frequency and duration of progeny collection (Table 1; online suppl. Table 1). Additionally, the misidentification in the double mutant genotype explained differences in expected recombination frequencies in these experiments (see below).

Two smaller pilot experiments had smaller sample sizes than Experiment 1 ($N = 9,755$) likely due to switching the cross design. These experiments helped to guide the approach in further experiments. For the double mutant stock, the overall haplotype frequencies were not significantly different from equal proportions (Table 2). Unlike the overall data, there were some significant haplotype

frequency skews that were most apparent at later time points and evident in both the control and high-temperature crosses (Table 3). Specifically, there was a bigger skew in the 2 recombinant haplotypes, with the *y +* haplotype being more frequent when frequencies were significantly different (Table 3). The most skewed proportions were found in the last time point on days 13–15 which had the fewest progeny. The next most skewed time point was the 7–9-day time period. Unlike the non-significant variation between total progeny in Experiment 1, investigation based on sexes led to noticeable variation for both mutant and wild-type haplotype groups, but more skewed in female progeny (online suppl. Table 9).

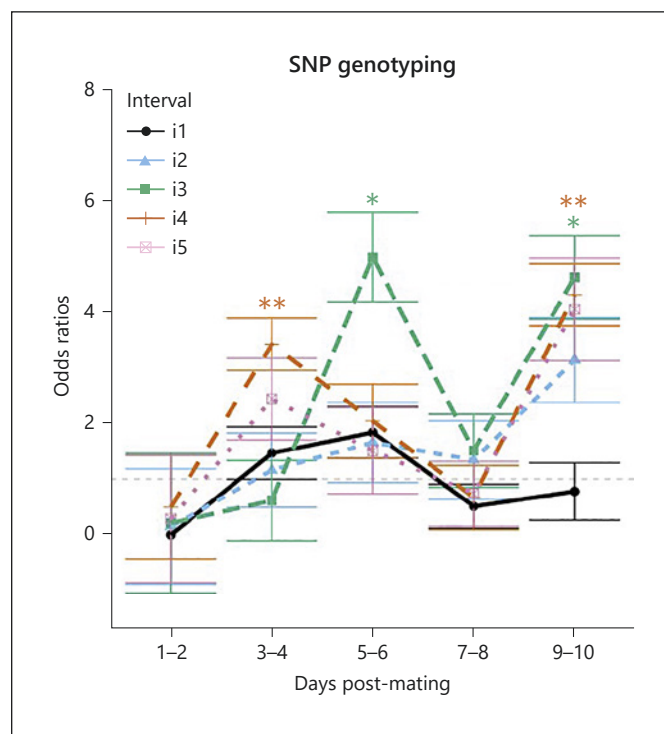


Fig. 5. Recombination results for SNP genotyping recombination rate analysis. Recombination frequencies between control and treatment were compared using a fitted model using logistic regression. SNP genotyping markers span 5 intervals that overlap the *y-st* and *sd-y* intervals (Fig. 1). In the overall model (Eq. 2) treatment was significant for intervals 3 (green) and 4 (orange) (online suppl. Table 3). Exponentiating the coefficients generated the odds ratio. Odds ratios were plotted against days post-mating and indicate the odds of having a crossover in high temperature compared to control. A post hoc test was done to calculate significance for each time point between treatment and control with significance indicated via asterisks (see online suppl. Table 5). See Table 1 and Fig. 3 for additional details regarding experimental design.

Fig. 4. Condition-dependent viability results. Each panel features overall viability differences due to condition for each crossover (CO) class. Here haplotype bias was calculated by taking the ratio between the 2 haplotypes in the same CO class. For comparison, ratios were set up to always be below 1. Raw results are presented in Tables 2 and 3. Here, variability among F_1 replicate crosses is shown. **a** For Experiment 1, due to having only 2 mutants, the NCO class has the largest difference in number of mutations per haplotype and the SCO class has an equal number of mutants between haplotypes. **b, c** For Experiments 2 and 3, which used a triple mutant stock, the SCO and DCO classes are both comparisons between 1 and 2 mutants. Whereas the NCO classes compare between 3 mutations and none. **d** Same as panels **b** and **c**, but for maternal age instead of heat stress. Additional dots are the outliers. NCO, non-crossover; SCO, single crossover; DCO, double crossover.

Triple Mutant Stock Reveals Strong Condition-Dependent Viability Selection

In Experiments 2–4, the triple mutant line was crossed to wild type with “treeline” chromosomal arrangement (SCI_12.2), and phenotypes at 3 mutant X-linked markers were recorded. For the triple mutant stock, the overall skew was much higher than in crosses with the double mutant stock (Table 2). Experiment 3 was most affected as a whole with a 2.47× difference in the proportion of NCO haplotypes (Table 2; $p = 0.0001$) and 60% of haplotype pairs significantly different from equal proportions (Table 3). For recombinants, haplotypes with 2 mutant markers were typically lower in frequency than the alternative haplotype, with the exception being the $+ y se$ haplotype which is on average 1.41× higher than the $++ sd$ haplotype (Table 2). This observation holds for all time points and treatments, with the exception being a 1.3× increase in the $sd ++$ haplotype in days 1–3 for Experiment 4 (Table 3). This result suggests that the *scalloped* phenotype may contribute more to the bias in haplotype frequencies than the other mutant markers (but see below).

For Experiments 2 and 3, more than double the time points were significantly skewed in the control temperature as compared to the high temperature crosses, whereas for Experiments 1 and 4 both treatments had a similar number of skewed frequencies across time points (Table 3). Additionally, for Experiment 2 the 7–9-day time period had the most skewed haplotype frequencies. For Experiment 3, the 7-day time point had the most skewed proportions between haplotypes, and the 9-day time point had the fewest skewed haplotype proportions. Finally, for Experiment 4, the day 1–3 time point had the most skewed haplotype frequencies, predominantly in the control crosses; whereas the skew in haplotype frequencies in the day 10–12 time point are exclusively in the maternal age crosses (Table 3).

Fecundity Differences Support Stress of Selected Treatments

Viability differences, described in the further section, will also influence estimates of fecundity. Even though, in Experiments 2 and 4, the selected treatment had a significant effect on fecundity (Table 1; online suppl. Fig. 1; online suppl. Table 8), with a decrease in the treatment group indicating the stress response from the higher temperature of 26°C and the maternal age of 35 days. This effect could be influenced by the scoring of recombinant haplotypes. Similarly, fecundity declined steadily throughout progeny collection, consistent with a single

mating event for these experiments. For Experiment 2, there was a 51% decrease in mean fecundity due to temperature ($p < 0.0001$, see Table 1; online suppl. Table 8) that was significant for all time points (online suppl. Fig. 1). For Experiment 4, average fecundity for females aged 7 days used for the control crosses (70.36) differed from females aged to 35 days (54.29). A post hoc mean contrast found that fecundity was significantly different between treatments for the 1–3-day time point ($p = 1.46E-4$) and the 7–9-day post-mating time point ($p = 0.013$).

In Experiment 3, average sample sizes from days 6–10 in the control and experimental conditions were 20.9 and 15.0, respectively ($p < 0.019$). Because the eggs laid by females on days 1–5 were discarded (Fig. 3), this sample size does not represent lifetime fecundity. Still, the sample sizes were significantly different on days 6, 8, and 9 (online suppl. Fig. 1).

Condition-Dependent Variation Suggests Viability Selection of Mutant and Wild-Type Alleles

When comparing the haplotype skew across time points and treatments, an interesting pattern emerges that sheds novel light on condition-dependent viability selection. For example, in Experiments 2 and 3, which had a significant overall reduction in sample size due to heat stress, the apparent skew is higher in control crosses as compared to high temperature crosses. One possible explanation is that the wild-type stocks, being inbred laboratory strains held in a constant environment over many generations, have had fixation of alleles that are unfit at higher temperatures. This hypothesis is supported by the excess of mutant NCO class progeny in Experiments 2 and 4 seen in the 7–9-day time point (shown in bold and italic in Table 3). Assuming all mutant markers are equally unfit, the NCO class should show the largest skew against wild type as it has either 3 mutants or none. This switch in haplotype skew suggests that the wild type is also experiencing viability effects in addition to the visible mutant phenotypes for this treatment and time point. To further support this hypothesis, the skew is greater in control crosses for the NCO haplotypes than the heat stress crosses (Fig. 4b). This is further supported by the above-mentioned skew in the SCO class where the $sd ++$ haplotype has fewer progeny than the alternate haplotype which contains 2 mutant markers (y and se ; SCO1 in Fig. 4b). This skew is also significant for control crosses but not high temperature crosses in Experiment 2 for days 1–3 and 7–9 and Experiment 3 on day 6 (Table 3). A loss of wild-type haplotypes at the higher temperature (due to homozygous wild-type alleles that are tempera-

Table 4. Male and female count data from mutant marker Experiments 2–4

Experiment 2										
Treatment	Days 1–3		Days 4–6		Days 7–9		Days 10–12			
	M	F	M	F	M	F	M	F		
21°C	1,376***	1,605***	1,168***	1,567***	863***	1,101***	733***	887***		
26°C	760	793	563***	725***	433	458	315***	455***		
Experiment 3										
Treatment	Day 6		Day 7		Day 8		Day 9		Day 10	
	M	F	M	F	M	F	M	F	M	F
21°C	966	1,048	441	397	749*	837*	630***	785***	639***	785***
26°C	487***	667***	296*	243*	442	472	296***	384***	563	628
Experiment 4										
Treatment	Days 1–3		Days 4–6		Days 7–9		Days 10–12			
	M	F	M	F	M	F	M	F		
7-day	2,590	2,614	1,825***	2,121***	1,201***	1,882***	603	673		
35-day	1,459	1,535	1,443***	2,094***	830***	1,111***	776	802		

The number of male and female progeny per treatment and time period are shown. For Experiment 1, backcross was done to mutant stock (Fig. 2a), so those results are split further by CO type in online supplementary Table 9. However, for the other 3 experimental crosses, the backcross stock was wild type. Therefore, female progeny were never homozygous for the X-linked recessive markers and thus have no CO type information. Similar to the haplotype skew analysis in Tables 2 and 3, a binomial test was used to test for a significant deviation from 50:50 ratio (indicated by asterisks). Shown in bold is the only case of a significant male bias in progeny.

ture-sensitive) could result in a reduced apparent skew in haplotype frequencies overall, leading to lower or no detectable bias in the high temperature treatment (Table 3; Fig. 4). For Experiment 4, the bias in the crosses with increased maternal age does not see this reversal in the 35-day flies, suggesting it is specific to temperature stress. Therefore, the results suggest that the wild-type stocks experience selection most at 26°C and 7–9 days post-mating. In Experiment 3, with 24 h transfers, the NCO skew is significant for all time points except day 9 in both control and high temperature crosses, and day 10 for 26°C (Table 3). Similarly, the difference in NCO haplotype bias between temperatures is less apparent (Fig. 4c), likely because it hones in on the time period 7–9 that is most skewed in Experiment 2. Together, these results suggest that mutational load of both mutant and wild-type stocks are interacting to generate a condition-dependent pattern of haplotype bias.

To further investigate, the male-to-female ratios were evaluated (Table 4; online suppl. Table 9). Based on the cross design which backcrossed to wild-type males in Ex-

periments 2–4, there is an expectation that the female progeny would exceed the male progeny if viability selection of the mutant markers were the reason for the haplotype skew. For Experiment 2 control, this is always true – males are significantly reduced as compared to females for all time points (Table 4). However, for 26°C, only time points 4–6 and 10–12 see significant female bias. Whereas time points 1–3 and 7–9 do not see any such bias. Similarly, for Experiment 3, there is a lack of female bias on days 8 and 10 at 26°C. For day 7, there is a significant excess of male progeny ($p = 0.025$) at 26°C. This reduction of females as compared to males in 26°C crosses supports a viability effect of wild-type alleles, consistent with the excess of mutant NCO progeny as compared to wild-type NCO progeny on day 7–9 in 26°C reported above. This result supports the presence of alleles that are unfit at 26°C in the wild-type stock. This phenomenon is largely absent from Experiment 4, where maternal age was varied instead of temperature. Specifically, time points 1–3 and 10–12 were lacking a female bias, but this was true for both the control and maternal age treatment, with no significant male bias.

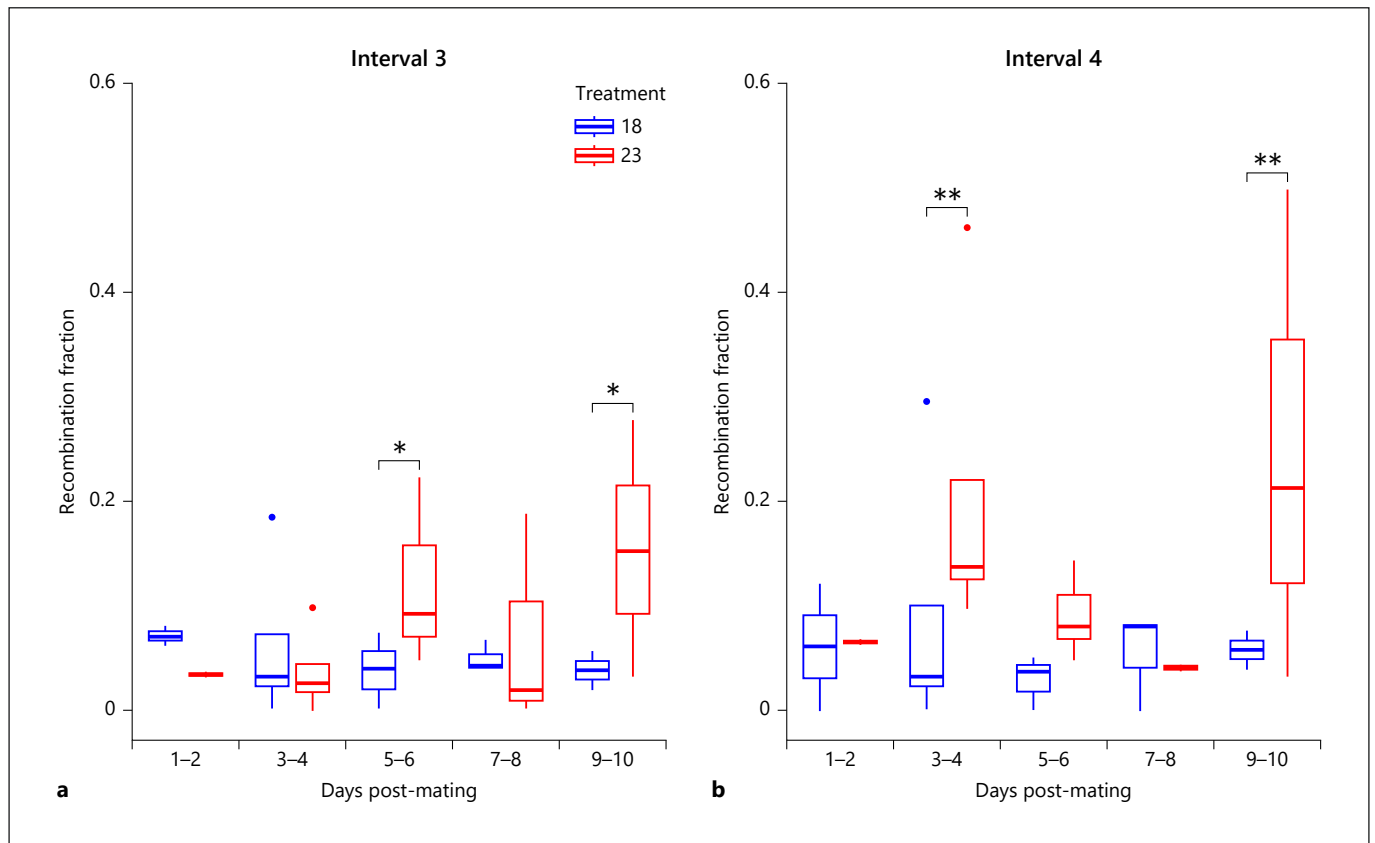


Fig. 6. Recombination rate differences due to heat stress in mutant and SNP genotyping crosses. Each panel shows individual box-plots of the variation in the Kosambi corrected recombination rate among the individual F_1 replicates per treatment. Significance between treatment and control for each time point in each plot is based on post hoc means contrasts and indicated by asterisks (see

online suppl. Tables 5 and 6). **a, b** Results from the SNP genotyping experiment for interval 3 (m_3 – m_4) and interval 4 (m_4 – m_5), which both show significant differences in recombination rate between control and high temperature treatment on days 9–10. Additional dots in red and blue are outliers.

Assuming this pattern is unique to the wild-type stock used in Experiments 2–4, a similar analysis was conducted on the Experiment 1 data, where male and female progeny were analyzed separately. Interestingly, among female progeny, the 25°C crosses had more mutant than wild-type NCO haplotypes on days 5–6 post-mating (shown in red in Table S9). For males, both treatment and control crosses had significantly reduced wild-type NCO progeny on days 7–9 and 10–12; whereas for 25°C, the time point 3–4 is also significantly skewed against wild-type progeny. This suggests the MV2-25 stock has similar fixation of alleles that are temperature sensitive, but at different time points and severity than the stock used in Experiments 2–4. Together, these findings suggest that the homogenous environment experienced by lab stocks fosters fixation of alleles that have lower viability across stressful environments (see Discussion).

SNP Genotyping Markers Reveal Recombination Plasticity of Temperature-Sensitive Time Points

In an earlier molecular analysis, results were described for markers on the 2nd chromosome [Stevenson et al., 2017]. That analysis also included 6 X-chromosome SNP genotyping markers in the region spanning the genes *yellow* and *vermillion* on the X-chromosome (Fig. 1b). In analyzing crossover data for intervals 1–3, the results show that control crosses had a 12.2% recombination rate, similar to the documented recombination fraction of 14.6 [Anderson, 1993]. The high temperature crosses had a 16% recombination rate across the same 3 intervals, which was significantly higher than the control ($p = 0.019$).

Across the 5 intervals, a significant difference due to temperature was observed for interval 3, between markers m_3 and m_4 , and interval 4, between markers m_4 and

m₅ (online suppl. Table 3). Additionally, a post hoc means contrast between treatment and control revealed a significant difference in recombination frequency in intervals 3 and 4 (online suppl. Table 5). Specifically, interval 3 differed on days 5–6, and interval 4 differed on days 3–4. Both intervals 3 and 4 had a significant peak difference on days 9–10 (Fig. 5, 6). Because intervals 3–4 overlap the *y st* and *y sd* regions, these SNP genotyping results are consistent with the sensitivity of recombination rate for similar time points (days 7–9 and day 9) and chromosomal regions as Experiments 1 and 3, respectively, that used mutant phenotypic markers (see online supplementary material). It is also worth noting that the magnitude of the difference due to temperature was higher for the SNP genotyping experiment than the experiments using phenotypic mutants (Fig. 5 vs. online suppl. Fig. 4).

Recombination Analysis of Mutant Markers Inconclusive due to Viability Effects

Despite the condition-dependent viability found here, these experiments were further investigated for differences in recombination frequency over time and due to treatment. Of course, this was done with the understanding that when haplotype frequencies are skewed (Fig. 4), an investigation of recombination frequency is flawed due to unrecovered haplotypes. Here, the average estimated proportion of missing progeny for double mutant experiments was 3.95% and for the quadruple mutant crosses was 19.45%. Therefore, a genotyping experiment using SNP markers was used to confirm the differences in recombination rate along a similar region of the X chromosome that the mutant phenotypic markers spanned (Fig. 1b; see below). For the triple mutant stock, results for the *sd y* region (32.1%) closely matched the expected rate (32.5%). Similar to *y st*, the *y se* region had a large recombination rate (46.0%) consistent with the genetic map distance (82 cM), since markers over 50 cM apart have a 50% recombination frequency (online suppl. Fig. 6a, b). Kosambi corrections indicate lower recombination rates across both intervals (*sd y*_{Kosambi} = 20.1%; *y se*_{Kosambi} = 40%), perhaps due to the lack of recovery of all progeny as evidenced by the skewed haplotype analysis above.

In Experiment 4, although treatment was not significant in the overall model, the interaction between time points and treatment was significant ($p = 0.02$; online suppl. Table 4) for the *sd y* interval. A post hoc mean contrast analysis revealed a significant difference in recombination rate ($p = 0.025$; OR = 1.16) in the first 72-h time point for the *sd y* interval (starred in online suppl. Fig. 4D, 6B; online suppl. Table 6). Although heat stress and ma-

ternal age indicate different time points as sensitive to recombination plasticity, these results are inconclusive due to the extreme skews in recovered haplotypes noted above.

Discussion and Conclusion

The present study represents a detailed comparison between the variability in haplotype bias and recombination rate variation in response to environmental stressors using consecutive experiments and several markers spanning the X chromosome. Even though previous work has extensively shown in *Drosophila* that heat stress led to recombination rate plasticity, the main focus was still on *D. melanogaster*. Our work was able to identify viability selection in the selected lines of *D. pseudoobscura* and analyze recombination rate plasticity using molecular SNP genotyping markers. Mutant phenotypic markers present rapid and inexpensive options for studying this phenomenon, yet are subject to viability selection. The results presented in this study confirm that environmental heterogeneity is a known source of fitness differences. Here, under heat stress, there was variation in the adherence to Mendel's first law using mutant markers which was shown to occur during similar time points where recombination rates were most sensitive using SNP markers.

Mutational Load May Lead to Condition-Dependent Viability Selection in Inbred Wild-Type Stocks

Meiosis is taught in introductory genetics classes to be highly predictable and reliable, and yet for years scientists have been puzzled by deviations from the expectations set out by Mendel regarding the segregation of alleles. While many studies investigate haplotype skew, or transmission distortion, for evidence of unfit alleles [Meyer et al., 2012; Fu et al., 2020], the role of the environment to alter this skew is often ignored [but see Shoben and Noor, 2020; Finnegan et al., 2021]. Environmental heterogeneity is a known source of fitness differences and yet, the adherence to Mendel's first law under various conditions has not been explicitly tested [Zwick et al., 1999; Finnegan et al., 2021]. Several studies have posited scenarios where competition among tetrads is variable across conditions suggesting recombination rate plasticity as a form of meiotic drive [Zwick et al., 1999; Haig, 2010; Stevison et al., 2017].

Biased haplotypes are a common observation when using mutant phenotypic markers, as certain genotypes are selected against due to viability effects, and are therefore not

recovered in the progeny [Hurst, 2019]. Still, they offer an inexpensive alternative to test a variety of conditions and time points, which is why they were used here. While our investigation into haplotype frequencies complicated the initial purpose of our investigation, our data provided a unique opportunity to explore how different temperatures impact haplotype frequency and point to increased mutational load in wild-type stocks. In this study, the segregation of the triple mutant gametes shows the greatest skewed haplotype frequencies in the progeny, seemingly driven by the *scalloped* locus. However, a more thorough investigation into these results led to the conclusion that the wild-type haplotype was being recovered with reduced frequency under high temperature stress across a selected number of time points. Interestingly, this points to a mutational load in the wild-type stock that is only revealed when reared at high temperatures. While the specific time points were not the same for the other wild-type stock, similar results suggest this could be a more common phenomenon among laboratory stocks of *Drosophila*.

While it is certainly not unexpected for wild-type stocks to harbor deleterious recessive alleles due to long-term inbreeding, these are infrequently tested for such prior to their use in experiments. Moreover, for those that do investigate for the potential of viability selection in mutant or wild-type stocks, this is likely only done in control conditions. Our results suggest that fecundity assays of wild-type stocks should be conducted across a range of conditions before use in experiments. This is especially true for experiments that aim to investigate stress, meiotic drive, or recombination frequencies. In fact, our cross design is ideal for uncovering such condition-dependent viability selection in wild-type stocks. For example, our design could be repeated with other wild-type stocks to examine the variation in this phenomenon across stocks. Further, our results suggest that fitness of lab stocks could be improved if they were reared under environmental heterogeneity to allow strains to purge unfit alleles that are sensitive across environments. This strategy should also be taken into consideration when establishing new lab stocks.

Experiments Point to Days 9–10 as Sensitive Period for Recombination Rate Plasticity

Similar to previous work [Stevison et al., 2017], we found a significant difference in recombination rate between flies reared at high temperatures as compared to control crosses for SNP markers on the X chromosomes. However, only the model tables for SNP genotyping intervals 3–4 were significant for treatment, whereas the

other experiments using mutant markers did not show a significant treatment effect (online suppl. Table 5). Further, post hoc analyses revealed various time points were significantly different between treatment and control with the most overlap between experiments on day 9 (9–10 in intervals 3–4 and 7–9 in Experiment 1; online suppl. Tables 5, 6). The results from the experiments using phenotypic mutants were complicated by apparent viability selection in both wild-type and mutant stocks, therefore, we focus our conclusions on the results from the SNP genotyping markers and heat stress. It is worth noting that the wild-type stocks used for SNP genotyping were different than the ones used for the crosses with the phenotypic mutants.

A sensitive period of 9–10 days closely corresponds to work in *D. melanogaster* which suggests a similar sensitivity around day 6 due to heat stress. In *D. melanogaster*, development from oogenesis to egg maturation takes 10 days. Oogenesis takes roughly a week and has been divided into 14 stages based on morphological criteria. Stage 1 is budding of the egg chamber from the germarium, and stage 14 is the mature egg. Oocyte selection and development during oogenesis occurs in stages 1–14 in the last 79 h [Koch and King, 1966]. Although, *D. pseudoobscura* oogenesis remains understudied, *Drosophila* species respond to temperature in a distinct manner. Still, a major benefit of *D. pseudoobscura* is the synchronicity of oogenesis among females that seems to alter with maternal age and indirectly affect fecundity (see Introduction). In *D. pseudoobscura*, eggs ripen as batches, with the immature eggs divided into groups of differing stages of development, ready to be deposited in large amounts at a time [Donald and Lamy, 1938]. Therefore, the number of eggs laid indicates a periodicity as compared to *D. melanogaster* that continuously lay their eggs in the 12-h day/night cycles.

In a series of experiments, Grell [1978a, 1984] was able to synchronize *D. melanogaster* eggs in age at the time of treatment, similar to the synchronicity observed in *D. pseudoobscura*. Her work identified variable expression of the gene *recombination defect* (*rec*) in temperature-sensitive mutants of *D. melanogaster*. The protein encoded by the *rec* gene, MCM8, is evolutionarily conserved and involved in generating meiotic crossovers and processive repair during DNA synthesis [Grell, 1978a, 1984]. MCM8 is transcribed in early developmental stages acting as a prerequisite for the formation of Holliday junctions and contributes to the stability of DNA strands during double-strand break and synaptonemal complex formation [Hunter, 2015]. In *Drosophila*, these events take place concurrently and affect regulation of crossovers [Carpenter

ter, 1975]. The protein complexes common in these processes show a temporal pattern that can be tracked by developmental stages. Grell's work in *D. melanogaster* showed that identifiable markers of DNA replication were present in the 16-cell cyst in the adult flies by 6 days, pinpointing the peak plasticity at the same time. To identify the peak timing of recombination due to temperature stress in *D. melanogaster*, 6-h transfers were conducted following perturbation [Grell, 1973]. While the experimental design in this study is quite different from Grell's work, it is worth noting that in *D. pseudoobscura*, late replication domains indicated with markers of repressive histone marks and SUUR protein are present in the early stages of oogenesis, indicating the pre-meiotic S-phase occurs after day 8 post-mating coinciding with the observed peak in recombination rate plasticity in this study [Grell, 1973; Higgins et al., 2012; Andreyenkova et al., 2013]. Because females were held for 7 days to sexually mature, the peak corresponds to 15–16 days post eclosion.

These similarities between species suggests that the physiological processes influencing recombination rate need to be further explored in a comparative context. Although there has been a lot of work done in *D. melanogaster*, there are other *Drosophila* species that may be more sensitive to environmental perturbations for studying this important phenomenon. Here, we have examined plasticity in the alpine species, *D. pseudoobscura*. Additionally, cactophilic [Markow, 2019] and mushroom feeding [Scott Chialvo et al., 2019] *Drosophila* represent recent adaptive radiations with growing potential for ecological genomics. Finally, the *montium* species group has recently become genome-enabled and is well suited for testing various evolutionary hypotheses [Bronski et al., 2020].

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Statement of Ethics

The authors have no ethical conflicts to disclose. Ethical approval is not required for this type of research.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Ulku Huma Altindag was responsible for data collection, data analysis, and writing of the manuscript. Laurie Stevison conceived and funded this project, as well as overseeing students. She was also involved in data collection, analysis, and writing the manuscript. Chelsea Shoben worked on the molecular verification of the *scarlet* mutant, as well as editing of the manuscript. Hannah Taylor helped with data collection for the mutant marker experiments, focusing on the maternal age experiments and editing of the manuscript. Keeley Pownall was involved in data collection for the mutant marker experiments and editing of the manuscript.

Data Availability Statement

Data files and scripts to complete the analysis are available on github at <https://github.com/StevisonLab/Peak-Plasticity-Project>. Zenodo was used to generate a DOI associated with a release of the code prior to publication: 10.5281/zenodo.6426328. This repository includes the raw survivorship data, raw mutant phenotype records for males, and female count data from the recombination analysis as csv files along with the R code. A separate csv file with treatment information includes dates and other metadata that would be needed to validate the analysis and conclusions herein. Additionally, a processed data file that includes sums of males and females, as well as crossover counts across each interval per time point, per replicate cross is also included. Also, a walk through the tutorial for the analysis of data in Experiment 1 in Rmarkdown has been included. Sanger sequence data for 179 bp of the *scarlet* gene, capturing the 2-bp deletion, in the bright red eyed flies (originally ordered *vermillion* stock) is available on NCBI (GenBank accession number MT438819). Additionally, a preprint version of this article is available on biorxiv [Altindag et al., 2020].

References

- Aggarwal DD, Rybnikov S, Cohen I, Frenkel Z, Rashkovetsky E, Michalak P, et al. Desiccation-induced changes in recombination rate and crossover interference in *Drosophila melanogaster*: evidence for fitness-dependent plasticity. *Genetica*. 2019;147:291–302.
- Agrawal AF, Hadany L, Otto SP. The Evolution of Plastic Recombination. *Genetics*. 2005;171: 803–12.
- Altindag UH, Shoben C, Stevison LS. Putative condition-dependent viability selection in wild type stocks of *Drosophila pseudoobscura*. *bioRxiv*. 2020.04.10:036129.
- Anderson W. Linkage map of *Drosophila pseudoobscura*. In: O'Brien SJ, editor. *Genetic Maps: Locus Maps of Complex Genomes*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1993. p. 3252–3.
- Andreyenkova NG, Kolesnikova TD, Makunin IV, Pokholkova GV, Boldyreva LV, Zykova TY, et al. Late Replication Domains Are Evolutionary Conserved in the *Drosophila* Genome. *PLoS One*. 2013;8:e83319.
- Beers CV. Linkage Groups in *Drosophila pseudoobscura*, Race B. *Genetics*. 1937;22:577–86.
- Bombliès K, Higgins JD, Yant L. Meiosis evolves: adaptation to external and internal environments. *New Phytol*. 2015;208:306–23.
- Bridges CB. The relation of the age of the female to crossing over in the third chromosome of *Drosophila melanogaster*. *J Gen Physiol*. 1927;8:689–700.
- Bronski MJ, Martinez CC, Weld HA, Eisen MB. Whole Genome Sequences of 23 Species from the *Drosophila montium* Species Group (Diptera: Drosophilidae): A Resource for Testing Evolutionary Hypotheses. *G3 (Bethesda)*. 2020;10(5):1443–55.
- Carpenter ATC. Electron microscopy of meiosis in *Drosophila melanogaster* females. I. Structure, arrangement, and temporal change of the synaptonemal complex in wild-type. *Chromosoma*. 1975;51:157–82.
- Charlesworth B, Barton NH. Recombination load associated with selection for increased recombination. *Genet Res*. 1996;67:27–41.
- Crew FAE, Lamy R. Linkage groups in *Drosophila pseudoobscura*. *J Genet*. 1935;30:15–29.
- Donald HP, Lamy R. VI.-Ovarian rhythm in *Drosophila*. *Proc R Soc Edinb*. 1938 VI;57:78–96.
- Dworkin I, Kennerly E, Tack D, Hutchinson J, Brown J, Mahaffey J, et al. Genomic Consequences of Background Effects on scalloped Mutant Expressivity in the Wing of *Drosophila melanogaster*. *Genetics*. 2009;181:1065–76.
- Finnegan SR, Mondani M, Fowler K, Pomiankowski A. Meiotic drive does not cause condition-dependent reduction of the sexual ornament in stalk-eyed flies. *J Evol Biol*. 2021; 34:736–45.
- Fu Q, Meng X, Luan S, Chen B, Cao J, Li X, et al. Segregation distortion: high genetic load suggested by a Chinese shrimp family under high-intensity selection. *Sci Rep*. 2020;10: 21820.
- Gloor GB, Engels WR. Single-Fly DNA Preps for PCR. *Drosophila Information Service*. 1992; 71:148–9.
- Grell RF. The Meiotic Origin of Temperature-Induced Crossovers in *Drosophila melanogaster* Females. *Genetics*. 1966;54:411–21.
- Grell RF. Recombination and DNA replication in the *Drosophila melanogaster* oocyte. *Genetics*. 1973;73:87–108.
- Grell RF. High frequency recombination in centromeric and histone regions of *Drosophila* genomes. *Nature*. 1978a;272:78–80.
- Grell RF. Time of recombination in the *Drosophila melanogaster* oocyte: evidence from a temperature-sensitive recombination-deficient mutant. *Proc Natl Acad Sci U S A*. 1978b;75: 3351–4.
- Grell RF. Time of recombination in the *Drosophila melanogaster* oocyte. III. Selection and characterization of temperature-sensitive and -insensitive, recombination-deficient alleles in *Drosophila*. *Genetics*. 1984;108:425–43.
- Haig D. Games in Tetrads: Segregation, Recombination, and Meiotic Drive. *Am Nat*. 2010; 176(4):404–13.
- Hales KG, Korey CA, Larracuente AM, Roberts DM. Genetics on the Fly: A Primer on the *Drosophila* Model System. *Genetics*. 2015; 201:815–42.
- Higgins JD, Perry RM, Barakate A, Ramsay L, Waugh R, Halpin C, et al. Spatiotemporal Asymmetry of the Meiotic Program Underlies the Predominantly Distal Distribution of Meiotic Crossovers in Barley. *Plant Cell*. 2012;24:4096–109.
- Hunter CM. *Environmental and Genetic Determinants of Recombination Rate in Drosophila melanogaster*. Ph.D., North Carolina State University; 2015.
- Hurst LD. A century of bias in genetics and evolution. *Heredity (Edinb)*. 2019;123:33–43.
- Koch EA, King RC. The origin and early differentiation of the egg chamber of *Drosophila melanogaster*. *J Morphol*. 1966;119:283–303.
- Kohl KP, Singh ND. Experimental evolution across different thermal regimes yields genetic divergence in recombination fraction but no divergence in temperature associated plastic recombination. *Evolution*. 2018;72:989–99.
- Kulathinal RJ, Bennett SM, Fitzpatrick CL, Noor MA. Fine-scale mapping of recombination rate in *Drosophila* refines its correlation to diversity and divergence. *Proc Natl Acad Sci U S A*. 2008;105:10051–6.
- Kulathinal RJ, Stevison LS, Noor MA. The Genomics of Speciation in *Drosophila*: Diversity, Divergence, and Introgression Estimated Using Low-Coverage Genome Sequencing. *PLoS Genet*. 2009;5:e1000550.
- Kuntz SG, Eisen MB. *Drosophila* embryogenesis scales uniformly across temperature in developmentally diverse species. *PLoS Genet*. 2014; 10:e1004293.
- Markow TA. Ecological and Evolutionary Genomics: The Cactophilic *Drosophila* Model System. *J Hered*. 2019;110:1–3.
- McGaugh SE, Heil CSS, Manzano-Winkler B, Loewe L, Goldstein S, Himmel TL, et al. Recombination Modulates How Selection Affects Linked Sites in *Drosophila*. *PLoS Biol*. 2012;10:e1001422.
- Meyer WK, Arbeithuber B, Ober C, Ebner T, Tiemann-Boege I, Hudson RR, et al. Evaluating the Evidence for Transmission Distortion in Human Pedigrees. *Genetics*. 2012;191:215–32.
- Modliszewski JL, Copenhaver GP. Meiotic recombination gets stressed out: CO frequency is plastic under pressure. *Curr Opin Plant Biol*. 2017;36:95–102.
- Noor MAF. Genetics of sexual isolation and courtship dysfunction in male hybrids of *Drosophila pseudoobscura* and *Drosophila persimilis*. *Evolution*. 1997;51:809–15.
- Page SL, Hawley RS. Chromosome Choreography: The Meiotic Ballet. *Science*. 2003;301: 785–9.
- Parsons PA. Evolutionary rates: effects of stress upon recombination. *Biol J Linn Soc*. 1988;35: 49–68.
- Phadnis N. Genetic architecture of male sterility and segregation distortion in *Drosophila pseudoobscura* Bogota-USA hybrids. *Genetics*. 2011;189:1001–9.
- Plough HH. The effect of temperature on crossing over in *Drosophila*. *J Exp Zool*. 1917;24:147–209.
- Plough HH. Further studies on the effect of temperature on crossing over. *J Exp Zool*. 1921; 32:187–202.
- Priest NK, Roach DA, Galloway LF. *Mating-induced recombination in fruit flies*. *Evolution*. 2007;61:160–7.
- R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2020. <https://www.R-project.org/>.
- Richards S, Liu Y, Bettencourt BR, Hradecky P, Letovsky S, Nielsen R, et al. Comparative genome sequencing of *Drosophila pseudoobscura*: Chromosomal, gene, and cis-element evolution. *Genome Res*. 2005;15:1–18.
- Ritz KR, Noor MAF, Singh ND. Variation in Recombination Rate: Adaptive or Not? *Trends Genet*. 2017;33:364–74.
- Samuk K, Manzano-Winkler B, Ritz KR, Noor MAF. Natural Selection Shapes Variation in Genome-wide Recombination Rate in *Drosophila pseudoobscura*. *Curr Biol*. 2020;30: 1517–28.e6.

- Scott Chialvo CH, White BE, Reed LK, Dyer KA. A phylogenetic examination of host use evolution in the quinaria and testacea groups of *Drosophila*. [Mol Phylogenet Evol.](#) 2019;130:233–43.
- Shoben C, Noor MAF. Variable fitness effects of *Drosophila* mutant markers across genetic backgrounds, temperatures, and containers. [Drosophila Information Service.](#) 2020;103:9–12.
- Singh ND. Wolbachia Infection Associated with Increased Recombination in *Drosophila*. [G3 \(Bethesda\)](#). 2019;9:229–37.
- Singh ND, Criscoe DR, Skolfield S, Kohl KP, Keebaugh ES, Schlenke TA. EVOLUTION. Fruit flies diversify their offspring in response to parasite infection. [Science.](#) 2015;349:747–50.
- Stevison LS, Noor MA. Genetic and Evolutionary Correlates of Fine-Scale Recombination Rate Variation in *Drosophila persimilis*. [J Mol Evol.](#) 2010;71:332–45.
- Stevison LS, Sefick S, Rushton C, Graze RM. Recombination rate plasticity: revealing mechanisms by design. [Philos Trans R Soc Lond B Biol Sci.](#) 2017;372:20160459.
- Sturtevant AH, Tan CC. The comparative genetics of *Drosophila pseudoobscura* and *D. melanogaster*. [J Genet.](#) 1937;34:415–32.
- Throckmorton LH. The phylogeny, ecology, and geography of *Drosophila*. In: King RC, editor. [Handbook of Genetics](#). New York: Plenum; 1975. p. 421–69.
- Zwick ME, Cutler DJ, Langley CH. Classic Weinstein: Tetrad Analysis, Genetic Variation and Achiasmate Segregation in *Drosophila* and Humans. [Genetics.](#) 1999;152:1615–29.