Refining the timing of recombination rate plasticity in response to temperature in *Drosophila pseudoobscura*

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Abstract

Meiotic recombination rates vary in response to environmental factors like temperature. Variation in recombination generates an additional source for genetic variation while errors in this pathway can lead to cancer and chromosome number anomalies. Estimating duration and sensitivity of a meiotic response to environmental perturbation requires an understanding of molecular events and well-designed experimental approaches. An earlier study suggested that the peak (most sensitive) timing of plasticity in *Drosophila melanogaster* occurred during the pre-meiotic interphase where DNA replication takes place in S-phase. Recently, heat stress has been shown to lead to plasticity in recombination rates in *D. pseudoobscura*. Here, a combination of molecular genotyping and a series of mutant screens were used to determine peak plasticity timing in this species. Mutant flies were reared in either control or stress temperatures in a traditional cross design. Using mixed model analysis, the odds of crossover formation was 1.53X higher during days 7-9 ($p<0.0017$) and 1.41X higher on day 9 ($p<0.022$) in high temperature as compared to control crosses, suggesting the time period as the timing of peak plasticity. Time of peak plasticity at day 9 in *D. pseudoobscura* can be explained by comparison to the model organism *D. melanogaster* due to similar timing of key meiotic events. This comparative approach will enable future mechanistic work on the effects of temperature stress on recombination rate.

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 gamete viability. Additionally, crossing over during meiosis creates novel genetic variation by recombining parental haplotypes, which can facilitate adaptation to novel environments. This novel genetic variation due to recombination has important consequences for adaptation of species (Charlesworth & Barton, 1996; Page & Hawley, 2003).

Early studies in Drosophila have shown that recombination rates vary as a result of exposure to various treatments including maternal age, starvation, humidity, and temperature (Plough, 1917, 1921; Bridges, 1927). Over the last century, other model systems have replicated these results (reviewed in (Parsons, 1988; Agrawal *et al.*, 2005; Stevison *et al.*, 2017)). While perturbations have consequences on events throughout meiosis suck as in synaptonemal complex and double strand break (DSB) formation, early meiosis appears to be most sensitive to perturbation (Stevison *et al.*, 2017). Experimental evidence points to temperature sensitive pre-meiotic interphase as the stage when recombination rate's plasticity is the highest (Grell, 1973). This coincides with the relationship between meiotic recombination and DNA replication at S-phase (Grell, 1973, 1978b). Grell's studies followed 6 hour transfers to identify the peak timing of recombination due to temperature stress in *D. melanogaster*, to occur at six days following perturbation (Grell, 1973).

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. Drosophila is an extremely diverse genus made up of over 2000 species that diverged over 50 million years ago (Hales *et al.*, 2015). Some have argued that *Drosophila* species can serve as indicators of

global climate change (Parsons, 1991). One concern with focusing on *D. melanogaster* in the study of how environmental stress impacts recombination is that it is a cosmopolitan species, and thus 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 *D. pseudoobscura* (Stevison *et al.*, 2017).

While traditionally studied for their inversion polymorphisms, *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). 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 burst 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.*, 2019).

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 our study, we found significant plasticity in eight 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 work done in *D. melanogaster* (Grell, 1966, 1973, 1978a).

Here, we continue our work to establish *D. pseudoobscura* as a model for studying recombination rate plasticity. Specifically, we present a series of experiments (summarized in Table 1) aimed at refining the sensitive time period when recombination rate maximally differs in response to temperature. We use a temperature treatment throughout development similar to

work of Plough and others (see Figure 2 in (Plough, 1917, 1921; Stevison *et al.*, 2017)). Our previous work used genotyping markers, which showed plasticity along the genome spanning selected mutant markers. The majority of the work described here involves mutant phenotype screens. Combining strategies used in earlier studies, the work we present here will be essential to future mechanistic work to understand the mechanism of recombination rate plasticity and enable it to be studied in more depth in *D. pseudoobscura*.

Materials and Methods

Stocks

Mutant screens were conducted using two X-linked recessive mutant *D. pseudoobscura* stocks. First, a double mutant stock was produced by crossing two lines obtained from the U.C. San Diego stock center (which has relocated to Cornell University): *vermilion (v*; 1-88.3*) (*stock 14011-0121.06, Dpse\v[1]*) and yellow (y*; 1-74.5*)* (stock 14044-0121.09, Dpse\y[1]). Our analysis found that the *vermillion* phenotype is most likely *scarlet* (see Results). Mutations of the *vermilion* (and *scarlet*) gene induce a bright red-eye phenotype, and mutations within the *yellow* gene induce a yellow-hued body and wings. Second, a quadruple mutant stock (courtesy of Nitin Phadnis) was used that had four mutations - *yellow* (*y*; 1–74.5), *cut* (*ct*; 1–22.5), *scalloped* (*sd*; 1–43.0), and *sepia* (*se*; 1–156.5) (Phadnis, 2011). Mutations of the *cut* and *scalloped* genes induce changes to the wing phenotype, whereas mutations in the *sepia* gene result in brown eyes. Genetic locations of all mutant markers are shown in Figure 1B. *ct* marker produced inconsistent results due to a variation in penetrance of the *cut* mutation (Dworkin et al., 2009). Therefore, we excluded the *ct-sd* region from the remainder of our analysis.

Three wildtype *D. pseudoobscura* stocks were also used for genetic crosses. First, MV2-25 was used with the double mutant stock, which are both in an Arrowhead 3rd chromosome background. Second, AFC-57 (collected in 2015 by K. Ritz from American Fork Canyon, Utah; see (Ritz *et al.*, 2017)) was used for indel genotyping. Finally, to match the 3rd chromosome inversion arrangement of the quadruple mutant stock, a second stock also 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).

Husbandry & Cross Design

All stocks were maintained at 21°C with a 12 hour light-dark cycle in an incubator. Flies were reared on standard cornmeal–sugar–yeast–agar media in polypropylene enclosures. For indel genotyping, all crosses were performed at 20°C in glass vials containing 6mL of corn syrup food. Virgin female VY flies (5-7 days old) were crossed with male AFC-57. Virgin F_1 females (5-7 days old) were collected and crossed with VY male flies. Resulting backcross progeny were phenotyped. Cross design for the SNP genotyping markers was described elsewhere (Stevison *et al.*, 2017).

Mutant Screens

Mutant and wildtype stocks were crossed, and the heterozygous F_1 females were reared in the treatment conditions throughout development (egg-to-adult). F_1 females were backcrossed to wildtype males to ensure no fitness effects associated with recessive mutant alleles. However, for Experiment 3, the backcross was done to the mutant stock. Progeny were screened for recombination frequencies between treatment and control. Crossing schemes for mutant screens are diagrammed in Figure 2 with details on each experimental design outlined in Table 1. Virgin

F_1 females were collected within 8 hours after eclosion from the pupal case and held in the control environment for 7 days until reaching full sexual maturity. Males were individually isolated 24 hours prior to crosses to avoid crowding-induced courtship inhibition (Noor, 1997). Backcrosses were conducted as single male and female crosses, with males discarded after 24 hours to prevent additional stress from male harassment (Priest, 2007). 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. To increase sample sizes, multiple backcrosses were conducted from each replicate. Transfer frequency and duration were varied to pinpoint the timing of peak differences in recombination rate due to temperature (see Table 1).

Except for Experiment 3, only male progeny were scored and if any female progeny were found to be mutant, the entire vial was discarded and the data removed. Mutant screens recorded each of the mutant traits independently in a single-blind manner. For Experiments 4-5, 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. Every effort was made to score all progeny. However, for Experiments 1-3 this was challenging due to sample sizes that exceeded expectations. For this reason, sample sizes in Table 1 are an underestimate, and fecundity comparisons may be unreliable (see Results). Data were entered in triplicate and compared until 100% concordant.

Molecular 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 wildtype flies (methods described previously, see Stevison *et al.* 2017). In addition to the chr2 results previously described, seven 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* (Figure 1A). One marker was excluded based on screens of F_1 progeny, where the marker was completely homozygous, leaving 6 total. Together, the five intervals span 5Mb of the XL and are located on scaffold chrXL_group1e of the *D. pseudoobscura* reference genome. Additionally, two indel markers were designed based on the *D. pseudoobscura* assembly v3.1, each under 25kb from the *vermilion* and *yellow* genes and differing in length between the two stocks (Table S1). DNA was isolated (Gloor and Engels, 1992) from a minimum of 88 flies for each parent stock and backcross progeny for PCR amplification. 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 also genotyped.

Statistical Analysis

Statistical analysis was performed using R v3.5.2 (R Core Team, 2018). Glm function was used to generate a fitted generalized linear mixed-effects model (GLMM). For each interval within each experiment, a logistic regression analysis with a mixed model was conducted in R. The basic model equation was:

Equation 1: $$R = V + D + T + D*T$$

R indicates the binary response variable of whether an individual offspring was recombinant or not based on the pair of mutant phenotypes over the screened region. V indicates the replicate vial ID. Individual progeny were summed per replicate vial per day and any vial with fewer than 10 progeny were removed to avoid stochasticity in recombination rate estimates. D indicates days post-mating of the F_1 female. Finally, T indicates the temperature in which the F_1 female

was reared. For the most part, our interaction terms were not significant, so we extracted individual odds ratios for each time point (Figure 3). For logistic regression, exponentiating the coefficients of GLMM generates the odds of crossover formation between experimental and control conditions.

Additionally, to measure the impact of stress due to our treatment, we conducted a linear regression analysis following a similar basic model equation:

Equation 2: $$F = V + D + T + D*T$$

Here, all variables are the same as Eq. 1, except the response variable, F, in this model is the total number of progeny, fecundity, for each time point. For each replicate vial, fecundity was summed over all crosses and divided by the number of crosses per replicate. The results of both models are summarized in Table 1 and Supplementary Tables 2-3.

Results

SNP Genotyping markers confirm recombination plasticity of region spanning mutant markers

In our earlier molecular analysis, we described results for markers on the 2nd chromosome (Stevison *et al.*, 2017). That analysis also included six X chromosome genetic markers in the region spanning the mutants, *yellow* and *vermillion* (Figure 1A). In analyzing crossover data for intervals 1-3 (shown in black in Figure 3A), we found 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 three intervals, which was significantly higher than the control ($p=0.019$). Across the five intervals, we observed

a significant difference in recombination frequency (RF) in intervals 2-4 on days 5-6 and 9-10. There was a peak difference in interval 3 on days 5-6 (Figure 3A). These results confirmed the suitability of this region for use in a mutant screen approach to refine the timing of recombination rate plasticity in response to temperature stress.

Initial mutant screens using double mutant stock narrow plasticity to 7-9 days

We conducted a series of three experiments to pinpoint the timing of differences in recombination rate by crossing a double mutant stock (*yellow* and "*vermillion*") to the wildtype genome line MV2-25 (Figure 2A). These experiments varied in transfer frequency and duration of progeny collection (Table 1).

In Experiment 1, we extended the timeframe of the SNP genotyping experiment from 10 to 20 days with 120 hour transfers (Figure 3B). These results did not show any statistically significant differences in RF (RF_{25}=47.8%, RF_{20}=44.7%). Due to our experiment having only a single mating event, the progeny sample sizes for days 16-20 were too small for further consideration. We also shifted the control temperature to the more standard rearing environment of 20°C (Smith, 1958). To maintain a 5°C difference between treatment and control, the temperature treatment was also shifted to 25°C (Table 1).

In Experiment 2, we reduced the collections to 15 days and conducted 24 hour transfers. We found several time points had a low sample size with too few replicates and missing data for either treatment or control. Therefore, we aggregated time points for our analysis (Figure 3C). Although we saw minor differences in recombination ($RF_{24.5}$=46.7%, RF_{18}=44.3%) in days 3-4 (OR=1.48) and 10-15 (OR=1.57), these were not statistically different.

Experiment 3 was much larger, with 48-72 hour transfers and backcrosses to the mutant stock rather than wildtype. This resulted in a significant treatment effect in our statistical model ($p=0.02$, Table 1). Additionally, for days 7-9 ($p<0.0017$; $N_{20}=466$; $N_{25}=407$) there was a statistically significant increased odds of observing a crossover at high temperature with $RF_{25}=55.4\%$ and $RF_{20}=45.7\%$ (Fig. 3D). Additionally, days 13-15 were significantly different, but with a large standard error (see Table S3) and small sample size with fewer replicates ($N_{20}=387$; $N_{24}=99$). Thus, we reduced the collection period to 12 days and focused on days 7-9. Rearing temperature did not significantly explain differences in fecundity (Eq. 2) across Experiments 1-3 (Table 1). However, Experiment 1 and 3 showed a decrease in the mean fecundity between temperatures of 17% and 19% respectively (Figure S1).

Molecular genotyping reveals *vermillion* mutant is actually *scarlet*

In the experiments described above, the recombination fraction calculated between the red eye and yellow body phenotypes were 46%, much higher than the published 14.6%, indicating that one of the mutants was not in the expected gene region. For the indel marker near the *yellow* gene, our results indicated an association between genotype and phenotype (recombination fraction 0/88). However, upon genotyping an indel marker near the *vermillion* gene, we discovered that the bright red eye phenotype did not associate with the indel marker among backcross progeny (recombination fraction 39/85).

We then tested whether a mutation in *scarlet (st*), another X-linked marker in *D. pseudoobscura*, causes the red-eye phenotype in the progeny. Genotyping confirmed that the bright red eye phenotype was 100% associated with an indel within 21.9kb of the *(st)* gene (recombination fraction 0/471). Sanger sequence analysis revealed a 2bp deletion within the *scarlet* gene of the bright red eyed flies of this strain, suggesting a frame-shift mutation within the *scarlet* gene is a

likely cause for this phenotype. Although there is not a consistent map location for *scarlet* in the literature in *D. pseudoobscura* (Beers, 1937; Mampell, 1943), it is consistently 30cM away from *sepia* (Figure 1B). This places it roughly 52cM away from *yellow*, consistent with our observed recombination rates.

Mutant screens using quadruple mutant stock pinpoint 9 day peak in plasticity

Once we confirmed the double mutant stock contained markers that were >50cM apart, we obtained a quadruple mutant stock to continue mutant screens (Figure 2B). We conducted two additional experiments to refine the timing of recombination rate plasticity.

Our results in Experiments 4-5 for the *sd-y* region (38.5%) closely matched the expected rate (32.5%). Similar to *y-st*, the *y-se* region had a large recombination rate (46.4%) consistent with the genetic map distance (82cM). However, the results for the *ct-sd* region (6%) were substantially lower than expected (20.5%), thus we excluded the *ct-sd* region from our analysis (see Methods). In Experiment 4, the highest odds ratios were observed on days 4-6 for the *sd-y* region (1.24) and on days 10-12 for the *y-se* region (1.107, Figure 3E), though none of these results were significant. Still, based on the results from Experiment 3 in days 7-9, we focused on this time period with 24 hour transfers. In Experiment 5, the highest odds ratio was observed on day 9 (1.41) in the *y-sd* region (Fig. 3F, $p=0.035$, $N_{21}= 630$ and $N_{26}=296$). The total recombination across the *sc-se* region was also significant on day 9 (Table S3, $p=0.022$). Similarly, the median RF across replicates for this time point was 26% at 21°C and 29% at 26°C (Figure 4).

In Experiments 4-5, temperature had a significant effect on fecundity (Table 1, Supplementary Figure 1), with a decrease in the treatment group. For Experiment 4, there was a 51% decrease in mean fecundity due to temperature ($p<0.0001$, see Table 1). In Experiment 5 average fecundity from days 6-10 in the control and experimental conditions were 20.9 and 15.0, respectively ($p<0.019$).

Discussion

Our results in Experiment 3 narrowed the timing of recombination rate plasticity in response to heat stress to 7-9 day post-mating. The double mutant stock was then found to have markers that were 52cM apart rather than our expected 14.6cM distance. Therefore, we used a quadruple mutant stock to further narrow the timing of the peak difference in recombination rate due to temperature as day 9 post mating in Experiment 5. Interestingly, declined fecundity due to heat stress was only significantly different in Experiments 4-5 (Figure S1) where the temperature stress was at 26°C (maximum of the species range, (Kuntz and Eisen, 2014). We also found a significant correlation between fecundity and recombination rate among the replicates for day 9 in Experiment 5 (Figure S2B) in both the control ($p_{Exp5}=0.04$) and treatment ($p_{Exp5}=0.02$). This was not the case for days 7-9 in Experiment 3 (Figure S2A).

Another interesting finding was that the region spanning the centromere and out into XR (*y*-*v*; *y*-*se*) appears to have a peak recombination difference due to temperature slightly earlier. This finding matches previous experiments in *D. melanogaster*, which showed that regions near the centromere were affected by varying environmental conditions (Grell, 1973). For example, the SNP genotyping had a peak odds ratio of 6.97 on days 5-6 for the region between m_3 and m_4 (Fig 3A). Similarly, in Experiment 5, the interval *y*-*se* had a peak odds ratio of 1.29 on day 6 (Figure

3F, NS), as compared to the *sd-y* which peaked on day 9 (Figure 4). This finding suggests the timing of plasticity varies spatially along the genome as well as over time, not unlike the findings of Grell (Grell, 1973). A more comprehensive genome-wide study would be useful to understand the genomic properties that contribute to this spatial variation.

Finally, we show that our results are reproducible by summing data over multiple days from the 24 hour experiments, we compared them to the multi-day transfers. Results between Experiments 4 and 5 were highly concordant and within the 95% CI for intervals *y-sd* and *y-se* (Figure S3). However, for the interval *y-st* between Experiments 2 and 3, they were not overlapping. This is likely due to issues with Experiment 2 given the lack of progeny in several time points (see Results).

Peak plasticity at day nine points to events in early meiosis

The physiological processes influencing recombination rate need to be further explored. 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. For example, cactophilic (Markow, 2019) and mushroom feeding (Scott Chialvo *et al.*, 2019) Drosophila represent recent adaptive radiations with growing potential for ecological genomics. Additionally, the *montium* species group has recently become genome-enabled and is well suited for testing various evolutionary hypotheses (Bronski *et al.*, 2020).

Here, we have examined plasticity in the alpine species, *D. pseudoobscura*. Exchange between homologous chromosomes starts in the early pre-meiotic stages and are not yielded as crossing over until metaphase I in all eukaryotes (Page and Hawley, 2003). In *D. melanogaster*, development from oogenesis to egg maturation takes 10 days and a fully matured oocyte is

292 selected in stages 1-14 in the last 79 hours (Koch and King, 1966). Although, *D. pseudoobscura*
293 oogenesis remains understudied, *Drosophila* species respond to temperature in a distinct manner.
294 Moreover, the decrease in developmental time due to increased temperature is muted in alpine
295 species such as *D. pseudoobscura* (Kuntz and Eisen, 2014). For example, in Experiment 4, we
296 saw a 3-4 day shorter development time in the high temperature treatment as compared to the
297 control.
298 Moreover, crossover formation varies in the same way as different phenotypes are influenced by
299 physiological factors. Developmental events such as invagination of the cephalic fold in stage 8
300 and retracting of the germband in stage 13 of oogenesis happen faster in high temperature in *D.*
301 *pseudoobscura*. The gene *recombination defect (rec)* was identified by Grell in temperature
302 sensitive mutants of *D. melanogaster*, is involved in generating meiotic crossovers, and DNA
303 double strand break (DSB) formation (Grell, 1984). Later studies in *D. pseudoobscura* examined
304 the protein encoded by the *rec* gene, MCM8 showed it was evolutionarily conserved. This
305 protein complex contributes to the stability of DNA strands during DSB and synaptonemal
306 complex formation, and is transcribed early in developmental stages (Hunter, 2015). In
307 *Drosophila* these events take place concurrently and affect regulation of crossovers (Carpenter,
308 1975). The protein complexes common in these processes show a temporal pattern that can be
309 tracked by developmental stages. Grell's work in *D. melanogaster* showed that all three
310 processes were present in 16 cell cyst in the adult flies by 6 days pinpointing the peak plasticity
311 at the same time. While our experimental design is quite different from Grell's work, it is worth
312 noting that in *D. pseudoobscura*, late replication domains are present in the early stages of
313 oogenesis, indicating the S-phase occurs after day 8 post mating coinciding with the observed

peak in recombination rate plasticity in this study (Higgins *et al.*, 2012; Andreyenkova *et al.*, 2013).

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Conflict of Interest

Authors state that there is no conflict of interest.

Data Availability

Data files and scripts to complete the analysis are available on github (https://github.com/StevisonLab/Peak-Plasticity-Project). Also, we have included a walk through tutorial for the analysis of data in Experiment 3 in R markdown.

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Figure Legends

Figure 1. Physical and genetic location of markers used to measure recombination frequency in *D. pseudoobscura*. (A) 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 covers 62% of XL, including the mutant markers *yellow* and *vermillion*. (B) Genetic map of X chromosome with location of mutant X-linked markers for recombinant screens. The majority of markers were located on the XL. It was later discovered that our presumed *vermillion* marker in experiments 1-3 was actually *scarlet* (also shown).

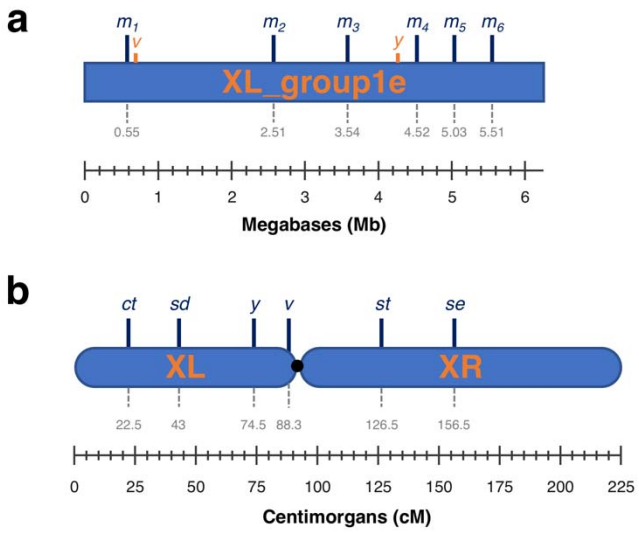


Figure 2. Crossing scheme for mutant phenotype screens. Females homozygous for mutant markers of two stocks were used to cross to wildtype flies. In Experiments 1-3, the *y-v* mutant stock and the MV2-25 wildtype stock were used (A). In Experiments 1 and 2, F_1 females were backcrossed to the wildtype stock, whereas in Experiment 3, F_1 females were backcrossed to the mutant stock (indicated by red asterisk). For Experiments 4-5, the quadruple mutant stock *ct-sd-y-se* and the SCI_12.2 wildtype stock were used (B). Based on initial screening of male progeny, the marker *ct* was removed from consideration as it gave unreliable results due to incomplete penetrance. Male progeny were screened for recombination analysis (Eq. 1) and female progeny were counted for fecundity analysis (Eq. 2).

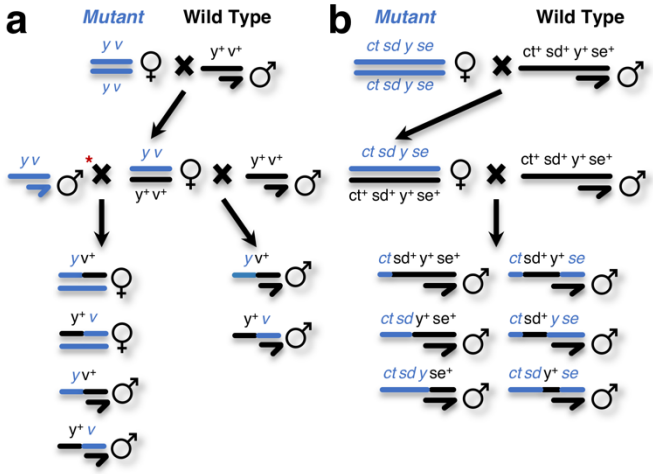
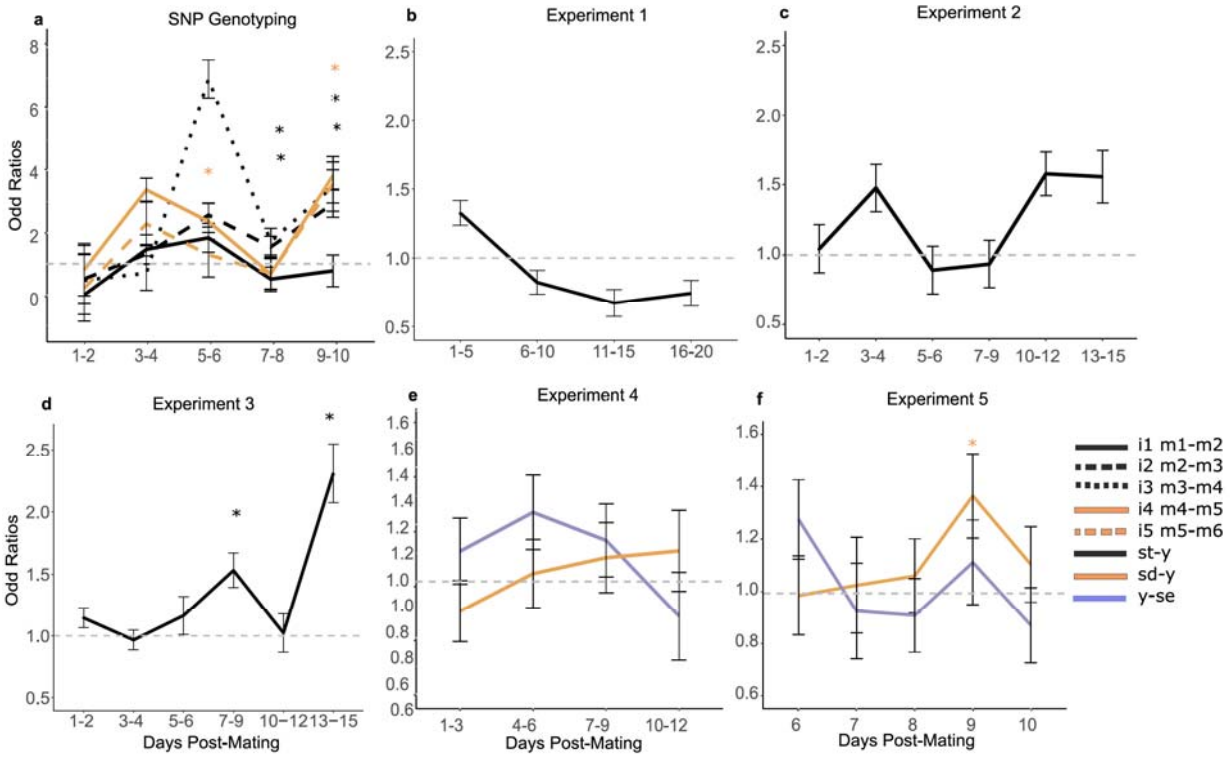
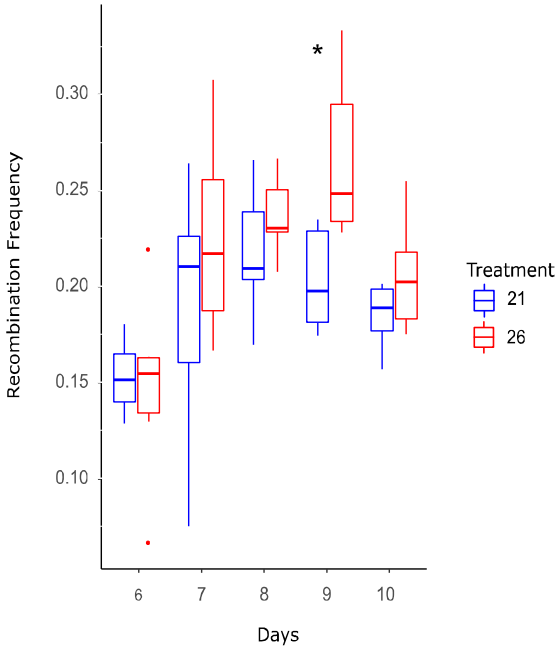


Figure 3. Recombination results for SNP genotyping and mutant screening Experiments 1-5. Recombination frequencies between control and treatment were compared using a fitted generalized linear mixed-effects model (GLMM). Exponentiating the coefficients generated the odds ratio. Odd ratios across experiments were plotted against days post-mating and indicate the odds of having a crossover in high temperature compared to control. (A) Odds ratios for the SNP genotyping markers. (B-D) Odds ratios for the double mutant screens in the *y-v* region, and (E-F) odds ratios for the quadruple mutant screens in the intervals *sd-y* (red) and *y-se* (blue). A post hoc test was done to calculate significance for each timepoint between treatment and control with significance indicated via asterisks (see Table S3). See Table 1 for additional details regarding differences in experimental design across panels.



452 **Figure 4. Recombination rates across interval *sd-y* over 24h periods from Experiment 5.** As
453 in Figure 3F, day 9 shows a significant difference in recombination rate (*p=0.035) between the
454 control (green) and high temperature (purple) for the interval between *scalloped* and *yellow*. The
455 boxplots show variation in recombination rate among the six replicates per treatment.



456

Table Legends

Table 1. Summary of experimental design for series of genotyping experiments to measure the impact of temperature on recombination frequency. For each experiment, a different set of temperatures, transfer frequencies and duration as well as sample sizes were used. *Note: Sample size is based only on the number of individuals targeted for recombination frequency. (e.g. only males were phenotyped for Exp 1, 2, 4, and 5). 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. For transfer frequency and duration, H denotes hours and D denotes days. P-value from Eq. 2 for Treatment on Fecundity. P-value from Eq. 1 for Treatment on Recombination Rate. Full anova tables for both analyses are in Table S2. [a]Crossing scheme matches Figure 2A. [b]Crossing scheme matches Figure 2B.

	Transfer frequency/duration	# rep vials/treatment	Median # crosses/rep	Temperatures	Sample Size*	Fecundity	Recombination
SNP Genotyping	48H/10D	4/4	4/4	18°C/23°C	677/611	n/a	0.29
Experiment 1[a]	120H/20D	4/5	8/6	20°C/25°C	678/529	0.36	0.71
Experiment 2[a]	24H/15D	4/4	9/8	18°C/24.5°C	1080/887	0.43	0.23
Experiment 3[a]	48H/6D; 72H/6-15D	8/9	10/10	20°C/25°C	5205/4488	0.24	**0.02**
Experiment 4[b]	72H/12D	5/5	5/5	21°C/26°C	4140/2071	**5.54E-08**	0.67
Experiment 5[b]	24H/6-10D	6/6	12/12	21°C/26°C	3425/2084	**4.49E-04**	0.88