Recombination Rate Plasticity in *Drosophila pseudoobscura*

Laurie Stevison[1], Ulku Huma Altindag[1], Hannah Taylor[1], Chelsea Shoeben[2], and Keeley Pownall[1]

[1]Auburn University, Auburn, AL 36830, [2]Duke University, Durham, NC 27705

INTRODUCTION

- In sexually reproducing organisms, meiosis enables ploidy reduction to generate gametes[6]
- In meiosis, recombination ensures proper segregation of chromosomes and generates novel genetic variation[6]
- For over a century, recombination rates (RR) have been demonstrated to be plastic due to a variety of intrinsic (e.g. age) and extrinsic (e.g. temperature) variables[9].
- Among fruit flies, this mysterious phenomenon has only been studied in *Drosophila melanogaster*[9]
- Here, we investigate RR plasticity in the alpine species[4] *D. pseudoobscura*

OBJECTIVES

- Determine peak timing of plasticity due to heat stress in *D. pseudoobscura*
- Determine if age impacts recombination rate in *D. pseudoobscura*
- Compare plasticity as a result of heat stress to plasticity due to maternal age

MATERIALS AND METHODS

Stocks

- A mixture of phenotypic mutant stocks and wildtype stocks were used.
- We first used a double mutant stock: *yellow* and "*vermillion*".
- After discovery that our red eye mutant was due to a mutation in the *scarlet* gene, we transitioned to a quadruple mutant stock: *cut*, *scalloped*, *yellow*, and *sepia*[7] (pictured on header).

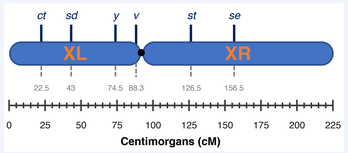
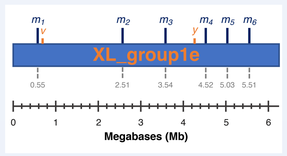


Figure 1. Genetic map of X chromosome with location of mutant X-linked markers for recombinant screens.

Molecular Genotyping

- Six SNP markers were design to overlap X-linked mutant markers[9].

Figure 2. Locations of SNP markers along scaffold "XL_group1e" located on the left arm of the X chromosome (XL), including the mutant markers *yellow* and *vermillion*.



- Two indel markers were designed near mutant markers *yellow* and *vermillion* to confirm genotype-phenotype association.

MATERIALS AND METHODS CONT'D

Mutant Screens for Recombination

- Mutant stocks were crossed to wildtype in a series of five experiments
- Experiments varied in transfer frequency and duration of egg laying period
- F1 females developed in either a control or treatment environment following methods of ref[8]
- Backcrosses to wildtype to reduce fitness effects
- Male progeny were screened for differences in RR between treatment and control

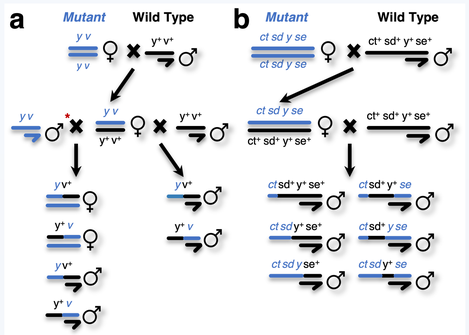


Figure 3. Crossing scheme for mutant phenotype screens. In Exp 1-3, the *y-v* mutant stock was used (a). In Exp 1 & 2, F_1 females were backcrossed to the wildtype stock, whereas in Experiment 3, F_1 females were backcrossed to the mutant stock (indicated by red asterisk). For Exp 4-5 and maternal age treatment, the mutant stock *ct-sd-y-se* was used (b).

Experimental Design

Table 1. Summary for genotyping experiments. *Sample size represents only males for all except Exp 3. Fecundity based on all progeny per replicate for the duration of each experiment. Significance of Treatment on ♀Fecundity and RR†.

	Transfer frequency/ duration	Control/ Treatment	# rep vials/ treatment	Median # crosses/ rep	Sample Size	Fecundity♀	RR†
SNP Genotyping	48H/10D	18°C 23°C	4	4	1,288	n/a	0.29
Exp 1	120H/20D	20°C 25°C	4	7	1,207*	0.36	0.71
Exp 2	24H/15D	18°C 24.5°C	4	8	1,967*	0.43	0.23
Exp 3	48H/6D; 72H/6-15D	20°C 25°C	8	10	9,693	0.24	**0.02**
Exp 4	72H/12D	21°C 26°C	5	5	6,211*	**5.54E-08**	0.67
Exp 5	24H/6-10D	21°C 26°C	6	12	5,509*	**4.49E-04**	0.88
Maternal Age	72H/12D	7 days/ 35 days	6	8	10,727*	**9.34E-04**	**2.96E-03**

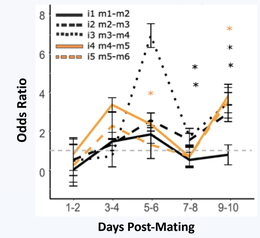
Statistical Analysis

- Logistic regression was used for RR analysis
  Equation 1: RR = V + D + T + D*T
- Linear regression was used for fecundity analysis
  Equation 2: Fecundity = V + D + T + D*T
- V=replicate vial; D=days post-mating; T=treatment
- Odds ratios were extracted from GLMM

TEMPERATURE STRESS ON RR PLASTICITY

SNP Genotyping markers confirm recombination plasticity of region spanning mutant markers

Figure 4. SNP genotyping results. RR between control and heat stress were compared using a generalized mixed-effects model in R. Exponentiating the coefficients generated the odds ratio. A post hoc test was done to calculate significance for each timepoint between treatment and control.



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

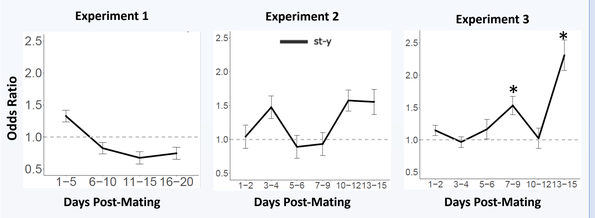


Figure 5. Exp 1-3 results. As in Figure 4, odds ratio vs. days post-mating are shown for mutant screens with *yellow* and "*vermillion*" stock.

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

Table 2. Indel markers were designed within 25kb of each gene and screened for recombination between the indel and mutants of the associated phenotype.

	yellow	*vermilion*	*scarlet*
Distance from indel to gene	23.2kb	3.3kb	21.9kb
Recombination Fraction	0/88 (0%)	39/85 (46%)	0/471 (0%)

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

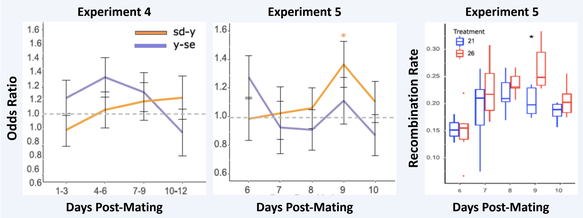


Figure 6. Exp 4-5 results. As in Figure 4, odds ratio vs. days post-mating are shown for mutant screens with quadruple mutant stock. The marker *ct* was excluded as it gave unreliable results due to incomplete penetrance. Shown right are RR results for the interval between *scalloped* and *yellow* for Exp 5.

Recombination Rate Correlates with Fecundity

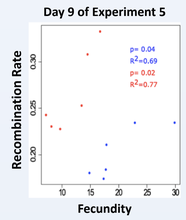
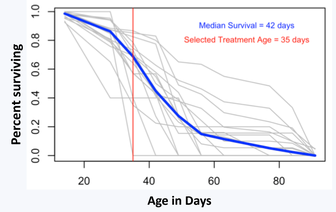


Figure 7. RR for the interval between *scalloped* and *yellow* versus fecundity among replicates from day 9 of Exp 5. Also shown is the p-value from the Pearson correlation test and the correlation estimate for both the treatment and control.

MATERNAL AGE ON RR PLASTICITY

Survivorship analysis guides choice of age

Figure 8. Survivorship curve of F_1 females from cross as in Figure 3. Eighteen replicate vials were tracked for ~3 months (each shown in grey) .



First evidence of impact of maternal age on RR in *D. pseudoobscura*

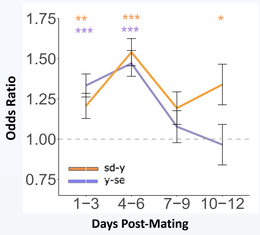


Figure 9. Maternal Age Results. Odds ratio vs. days post-mating are shown for maternal age experiment using quadruple mutant stock. Unlike Experiment 3, significant differences in RR occur at multiple time points in both intervals.

CONCLUSIONS

- RR peaked at days 7-9 post-mating in Exp 3
- 24H transfers narrowed the peak further to 9 days post-mating in Exp 5
- Comparison to timing of plasticity in *D. melanogaster*[2,10] and similar timing of meiotic events point[1] to this result being a result of perturbation of events in early meiosis[3,9]
- Maternal age has previously been shown not to influence RR in *D. pseudoobscura*[5]; however, we show that a 35 day age treatment does alter RR
- Unlike temperature, difference in RR due to maternal age peaked early and persisted over multiple time points, suggesting a permanent alteration of RR as a result of age

REFERENCES

1. Andreyenkova NG et al. (2013). PLOS ONE 8: e83319.
2. Grell RF (1966). Genetics 54: 411–421.
3. Grell RF (1978). Proc Natl Acad Sci 75: 3351–3354.
4. Kuntz SG, Eisen MB (2014). PLoS Genet 10.
5. Manzano-Winkler B, McGaugh SE, & Noor MAF. (2013). PLoS ONE: 8(8), 1–6.
6. Page SL, Hawley RS (2003). Science 301: 785–789.
7. Phadnis N (2011). Genetics 189: 1001–1009.
8. Plough HH (1917). J Exp Zool 24: 147–209.
9. Stevison LS et al. (2017). Philos Trans R Soc B Biol Sci 372: 20160459.
10. Sturtevant AH, Tan CC (1937). J Genet 34: 415–432.

ACKNOWLEDGEMENTS

Work supported by start-up funds to LSS from Auburn University. UHA was supported by NSF-DEB EAGER No. 1939090 to LSS. We thank members of the Noor Lab for generating the stock used for Exp 1-3. We thank Mohamed Noor for guidance and feedback. We thank the Graze lab for help with fly husbandry for Exp 1-3, which were conducted in the fly lab of Rita Graze. We thank the Phadnis Lab for sharing the quadruple mutant stock used for Exp 4-5. We thank Todd Steury for consultation on our statistical analysis. We thank members of the Stevison Lab for extensive help with mutant phenotype screens over a three year period.