Determining the Impacts of Transposable Elements on Recombination Frequency in Drosophila melanogaster

Background

Transposable elements (TEs), or transposons, are DNA elements with the ability to copy themselves to different locations within the genome. The transposition of TEs can disrupt gene function and reduce host fitness (Choi and Lee 2020). Because the transposition of TEs can often lead to unfavorable consequences, a host cell can induce repressive epigenetic marks onto its DNA in order to silence them (Slotkin and Martienssen 2007). However, these modifications can also spread to nearby genes, usually within 10 kb, causing them to become silenced as well (Choi and Lee 2020). There are two different classes of TEs, where each class is denoted by the mechanism by which the TE transposes. Class I TEs, or retrotransposons, are copied in 2 stages. DNA is transcribed into RNA and then reverse-transcribed into DNA, and finally inserted back into the genome. Class II TEs, or DNA transposons, are copied via a cut and paste mechanism to transpose and replicate (Wells and Feschotte 2020).

In our study, we propose a relationship between the presence of transposable elements in euchromatin and the recombination frequency in that region. Recombination frequency, or recombination rate, is the probability of a meiotic crossover event occurring between homologous chromosomes, resulting in a recombinant chromosome, which is then passed on to the offspring. Genetic recombination is a key process in meiosis that ensures proper segregation of chromosomes (Sherratt et al. 2004). It also has important implications for evolution because it can shape genotypic diversity (Cutter and Payseur 2013). However, little is known about the role of TE's in contributing to recombination frequency variation along the genome. Previous studies have shown that there is a negative correlation between TEs and meiotic recombination rates. The strength of this correlation varies depending on the TE element type (Kent, Uzunović, and Wright 2017). Here, we suggest that TEs play a direct role in determining local recombination rates.

It is known that in pericentromeric regions of plant chromosomes, recombination is suppressed due to the enrichment of H3K9me2 and non-CG DNA methylation (Underwood et al. 2018). The enrichment of these epigenetic marks cause the DNA to condense, making them less accessible. Without this mechanism in place, recombination occurring at or near the centromere can result in double-stranded breaks, leading to missegregation during meiosis and aneuploidy in the offspring. The suppression of recombination in these regions is therefore important for fertility in plants (Underwood et al. 2018).

We hypothesize that TE-mediated heterochromatin enrichment can lead to suppression of local recombination around TEs by a similar mechanism. Much like how enrichment of H3K9me2 and non-CG DNA methylation causes DNA in pericentromeric regions to be less accessible, and thus suppresses recombination, we suggest that TE-mediated heterochromatin enrichment causes euchromatic regions within 10 kb of the TE insertion to be less accessible as well, and therefore

less susceptible to recombination. As such, the observed local recombination frequency will be lower than expected.

In this experiment, I will use two different TE elements to test this relationship in Drosophila melanogaster: Roo and 1360. Each element is representative of the two different classes of TEs where Roo is a Class I TE, or retrotransposon, and 1360 is a Class II TE, or DNA transposon. Retrotransposons have been shown to have stronger induced heterochromatin effects than DNA transposons (Lee and Karpen 2017). Therefore, recombination frequencies are also expected to be different between the two elements.

Objective

Our experiment aims to determine the recombination frequency in the same euchromatic regions of 3 different lines containing no or different types of TEs. We propose that recombination is likely to be suppressed in areas with transposon-induced formation of heterochromatin. We also take into consideration the effects of different types of TEs on recombination rate.

Approach

A line is defined as a set of genetically identical individuals. I will use three Drosophila transgenic lines, each with one of the following constructs: No-TE, 1360, and Roo. Each of these constructs will also be located within the same genomic location to ensure that the recombination rate measured is of the same DNA. The only variable factor is the presence or absence of TEs. The No-TE genotype will act as the control for determining the expected recombination rate in the absence of TEs and their epigenetic effects. Because Roo is a retrotransposon and is expected to induce the most heterochromatin formation in surrounding DNA (Lee and Karpen 2017), its recombination rate is also expected to be the lowest of the 3 genotypes.

Each construct contains one line with a mCherry gene serving as a visible marker and another line with a black marker, b, which is phenotypic recessive. These two markers are both located on Chromosome 2 within the genome and their various recombinations will be measured in the F2 offspring to determine the recombination frequency. The existing full genome recombination map estimates these two markers at around 7.5 cM apart (Comeron, Ratnappan, and Bailin 2012). This suggests an expected 7.5% of recombinant offspring.

The first marker, b, is phenotypically recessive and causes the fly to appear blacker than wildtype. The second phenotypic marker, mCherry, exhibits phenotypic dominance and appears as a red fluorescence when viewed under a fluorescent microscope. These phenotypic markers will be scored and flies will then be separated into the 4 possible phenotypes for each genotype.

Figure 1 details the designed cross for this experiment. The crosses will be the same across all genotypes. The parental generation will consist of females homozygous for the b marker crossed with males containing one mCherry marker. F1 female virgins expressing the mCherry marker will then be collected and backcrossed to males homozygous for the b marker. In the absence of

recombination, the F2 progeny are expected to display either phenotypic blackness or red fluorescence. In the case of recombination, we expect flies to exhibit both phenotypic blackness and red fluorescence or neither phenotype.

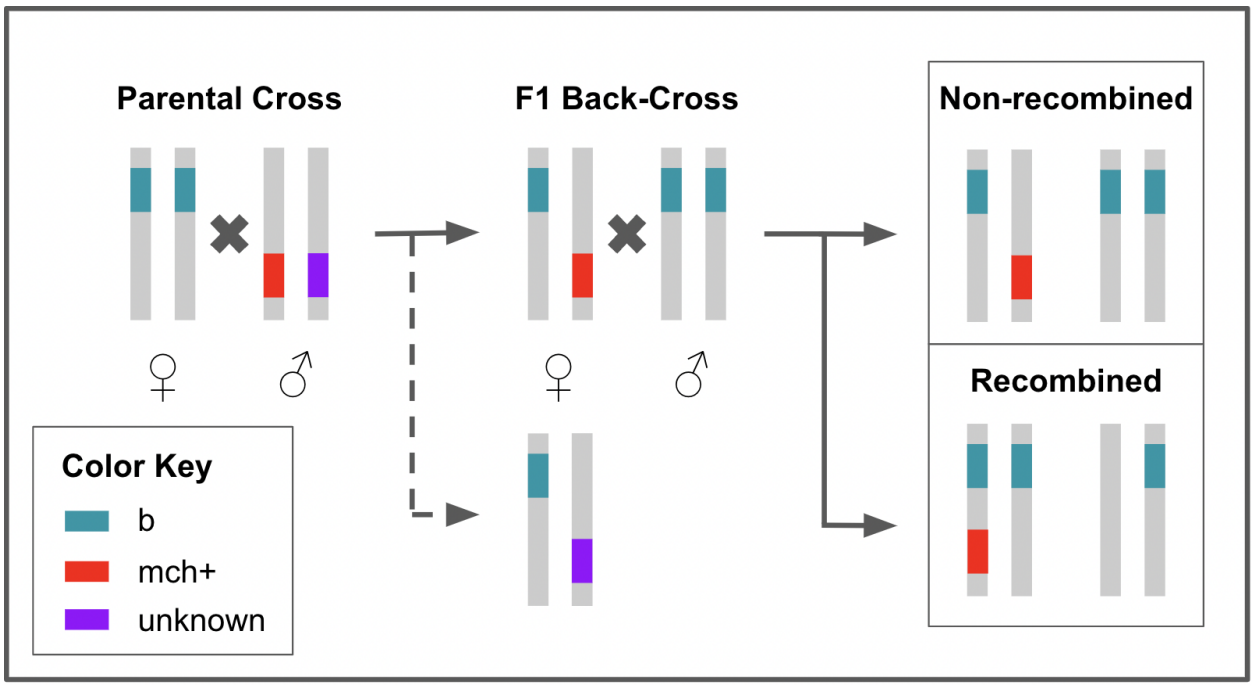

Figure 1. *Outline of crossing scheme and expected non-recombined and recombined results in F2 progeny. Only female flies exhibiting red fluorescence will be selected for the F1 back-cross from the F1 progeny.*

It is important to note that recombination can be influenced by age. This effect can vary, either increasing or decreasing recombination frequency. We will take account of this in our experiment and only compare individuals of similar ages across genotypes.

Outcomes and Predictions

We predict that the recombination rates in both the 1360 and Roo genotypes will be lower than the No-TE genotype due to TE-mediated heterochromatin enrichment leading to suppressed recombination. Additionally, Roo is expected to have the lowest recombination frequency of the 3 genotypes because it has the greatest induced heterochromatin enrichment (Lee and Karpen 2017). The expected recombination rate pattern is: No-TE > 1360 > Roo.

We have previously conducted this experiment in the summer of 2022 and found unusual results on the first day of scoring and counting individuals. Recombination frequencies for all genotypes were higher than expected. However, the following days of scoring and counting led to our predicted results (Figure 2). This outlier in the data may have been due to a technical error while

scoring the F2 generation. We also cannot rule out age effects as a possible cause. Because of this, we will repeat the experiment.

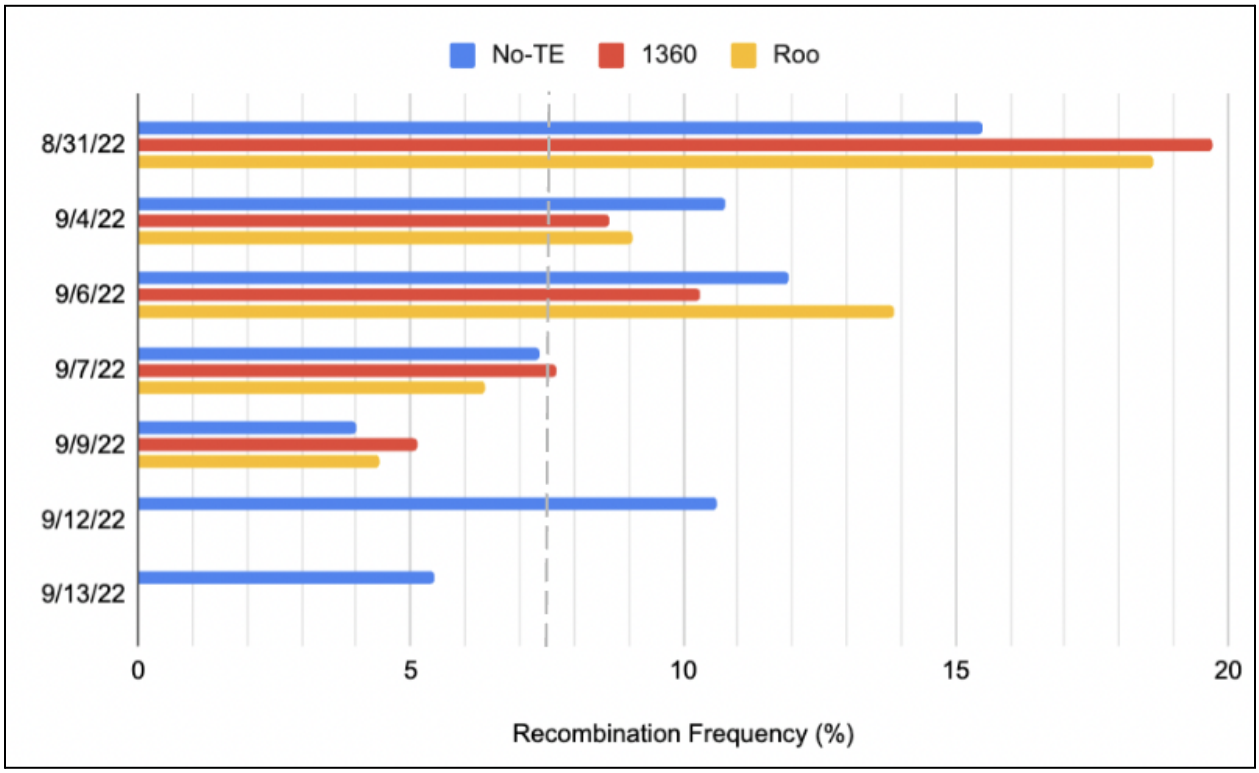

Figure 2. *Recombination frequency data collected from initial findings. The dashed vertical line at 7.5% denotes expected recombination frequency. Roughly 2000 F2 flies were collected from each genotype. No flies were collected for both 1360 and Roo on 9/12&9/13. The first day of scoring showed an unusually high recombination rate for all genotypes, serving as an outlier to the data. The averages for the No-TE, 1360, and Roo genotypes, respectively, were 9.86, 10.52, and 9.03%. Excluding results from 8/31, the average values decrease to 8.79, 8.18, and 7.85%.*

Specific Responsibilities

For each of the 3 genotypes:

- Collecting female virgins and setting up crosses/backcrosses
- Scoring phenotypic markers under the microscope/fluorescent microscope
- Counting individuals from each of the four possible phenotypes

This project will be carried out with the help and guidance from my Principal Investigator and a Postdoctoral Scholar

Timeline

- Oct. 1 to Oct. 23: Expand each genotype stock
- Oct. 24 to Nov. 12: Set up parental crosses for all genotypes

- Nov. 13 to Dec. 3: Set up F1 backcrosses for all genotypes
- Dec. 3 to Dec. 24: Score phenotypic markers in F2 generation
- Jan. 5 to Jan. 30: Data analysis
  Note: Each generation time is approximately 2 weeks and additional time is needed to ensure the crossing females are virgins.

References

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Itemized Budget

Quantity	Item Description	Unit Price	Total Price
12	Fly Food Trays	$45	$540
5	Cotton Ball Bags	$50	$250
7	CO_2 Tanks	$30	$210
1	Presentation Poster	$150	$150

TOTAL: $1150