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Genetic Factors Affecting Hybrid Male Sterility Leading to Speciation

Patrick Opperman
University of Nebraska - Lincoln, pjopperman0@gmail.com

Colin D. Meiklejohn
University of Nebraska-Lincoln, cmeiklejohn2@unl.edu

Genetic Factors Affecting Hybrid Male Sterility Leading to Speciation

Patrick Opperman and Colin Meiklejohn, Ph.D.

School of Biological Sciences, University of Nebraska – Lincoln, NE - 68588

ABSTRACT

The process whereby speciation occurs can come about through the evolution of barriers to gene flow. One of these barriers to gene flow can be an incompatibility, which leaves hybrids dead or sterile. Two theories underlie the work of this experiment, Haldane's Rule and the large X effect. Haldane's Rule is the observation that unisexual inviability or sterility among species' hybrids is almost always found in the heterogametic sex. The large X effect is the observation that substitution of one species' X-chromosome for another's has a disproportionately large effect on hybrid fitness compared to similar substitution of an autosome. For Drosophila, the cause of the large X effect has been identified as density dependent for the number of sterility-inducing incompatibilities on the X-chromosome. In this project we are using *Drosophila simulans* and *Drosophila mauritiana*. We are using introgressed lines of flies that make use of physical markers that can be used to track the progress of genetic material throughout crosses. The visible markers that we are using affect eye color and express fluorescent protein, allowing us to determine the regions on the recombinant chromosomes that contain the factors leading to hybrid male sterility. Males that carry the recombinant X-chromosomes are sterile unless the sterility factors have been removed via recombination. Flies that are fertile will be genotyped using Real-Time PCR. Genetic mapping will then allow us to determine the location of the sterility-causing gene in question. At this time we have generated a number of recombinant genotypes and through the genotyping of these samples we have narrowed our candidate region. At the start of this project the region was approximately 300kb in length and we have shortened that segment of interest to 100kb. By shortening this segment we have narrowed our search area for this sterility-causing gene that is of interest to us.

INTRODUCTION

Speciation occurs when two populations become reproductively isolated from each other through the evolution of one or more barriers to gene flow. Genetic incompatibilities can render hybrids sterile or dead and are one form of reproductive barriers. Previous genetic studies have revealed an important role for the X chromosome in the evolution of hybrid incompatibilities. The role is indicated by Haldane's Rule, which is the observation that unisexual inviability or sterility among species' hybrids is almost always found in the heterogametic sex[1]. It is known that the large X effect – the observation that substitution of one species' X-chromosome for another's has a disproportionately large effect on hybrid fitness compared to similar substitution of an autosome – can at least partially explain Haldane's Rule. In Drosophila, the primary cause of the large X effect has been identified as a higher density of hybrid incompatibility loci on the X-chromosome than the autosomes[2]. Sterility-inducing incompatibilities evolve early during speciation thus the changes in spermatogenesis that lead to the inability of hybrids to reproduce is of keen interest. The goal of this project is to use genetic mapping of X-linked factors that cause hybrid male sterility to determine the location and identity of the underlying genes leading to speciation.

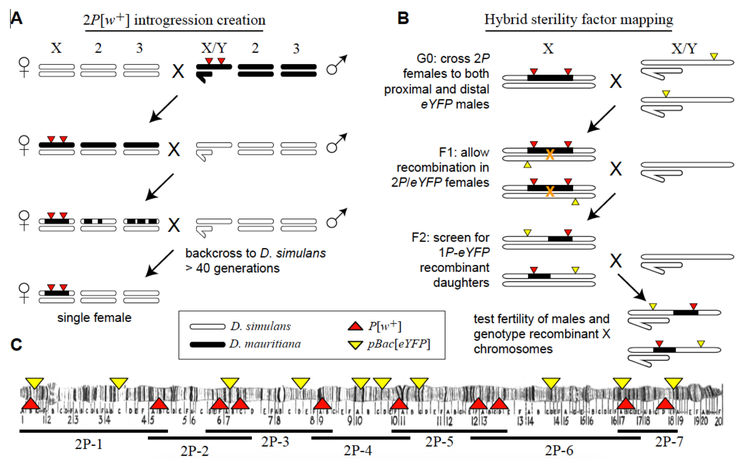
For this project, we have focused on two species, *Drosophila simulans* and *Drosophila mauritiana*. Previous research in the lab has demonstrated the existence of at least seven X-lined genetic factors causing male sterility in hybrids between these species, and our plan is to determine their exact position. We will be using introgression lines of flies in this project. Introgression involves crossing small segments from the *D. mauritiana* genome into the *D. simulans* genome. These lines have specific segments from *D. mauritiana* on the X-chromosome and these areas signify where to look when interpreting the results of our genetic mapping. These lines are central to this research in determining the genetic factors that lead to speciation.

METHODS

The introgression of specific segments of DNA from *D. mauritiana* into *D. simulans* is made possible with P-elements inserted across the X-chromosome. The visible markers we are using affect eye color and express fluorescent protein, which allows us to determine the regions on the recombinant chromosomes containing the factors leading to hybrid male sterility. The introgressed segments from *D. mauritiana* initially carry two P-elements affecting eye color (2P) and show a dark orange/red eye color. When one P-element is removed by recombination 1P flies allow us to track *D. mauritiana* genetic material.

A second type of P-element insert that causes flies' eyes to glow fluorescently when illuminated by blue light is used to identify regions of the X-chromosome from *D. simulans.* When X-chromosome segments are subdivided by recombination, males will have a light orange eye color (1P) and will glow fluorescently. Recombinant flies were genotyped using Real-Time Polymerase Chain Reaction (qPCR). Genetic mapping will allow us to determine the location of sterility-causing genes. Using the published genome sequences for *D. simulans* and *D. mauritiana* we will use the information to take our genetic mapping results and identify candidate genes or gene regions that cause sterility in order to identify the factors that lead to the evolution of reproductive barriers.

Fig. 1 Introgression of P elements in the X chromosome



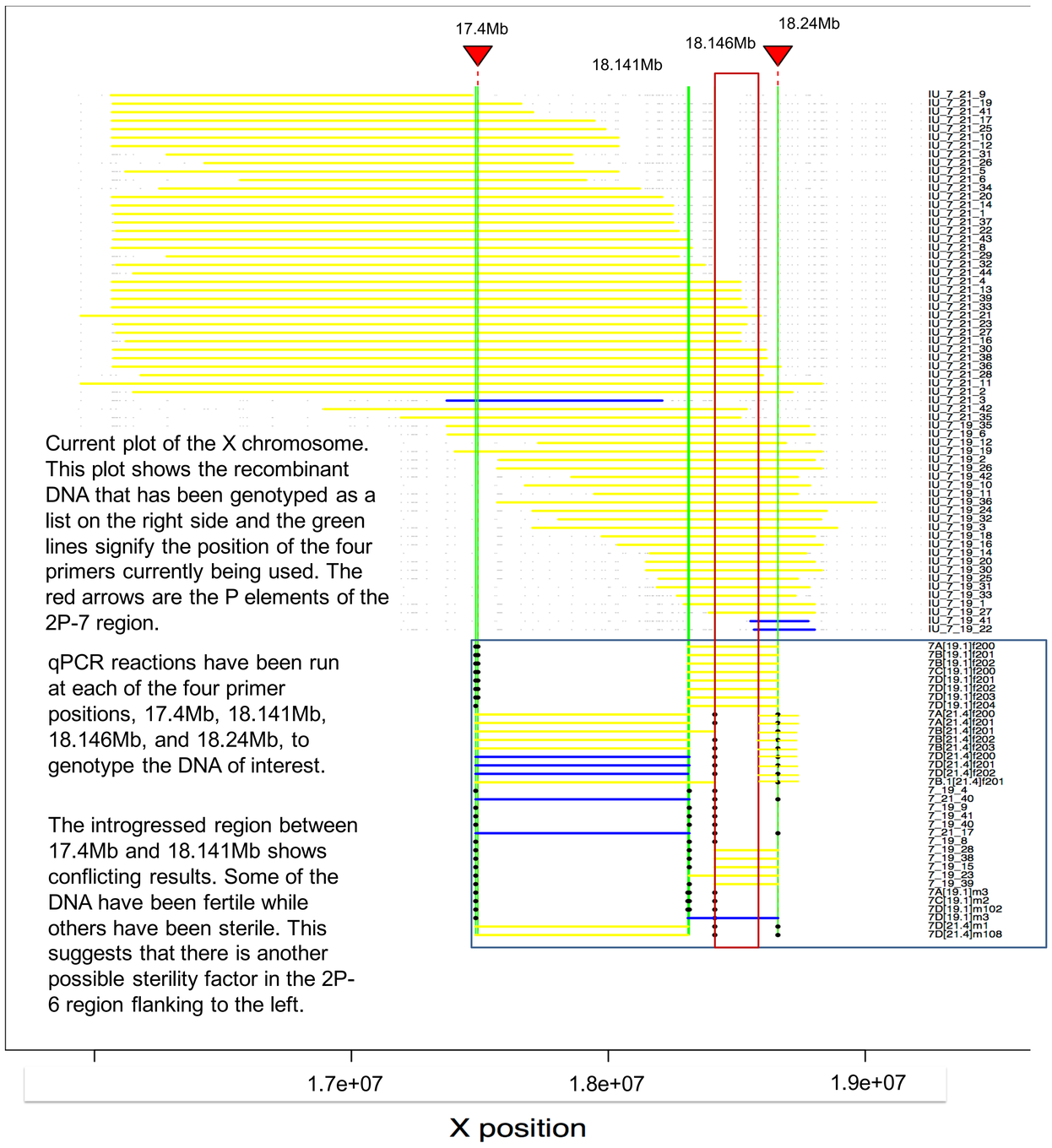
A. This figure shows the method used to create the lines of interest for this research project, introgression lines. The lines with the P elements are utilized to help track segments of DNA.

B. Crosses displaying the methods used to generate recombinant flies for genotyping.

C. Mapping of the seven P element regions used on the X chromosome. My project focuses on the 2P-7 region of the chromosome.

RESULTS

Fig. 2 X Chromosome Plot of Genotyped Recombinant DNA

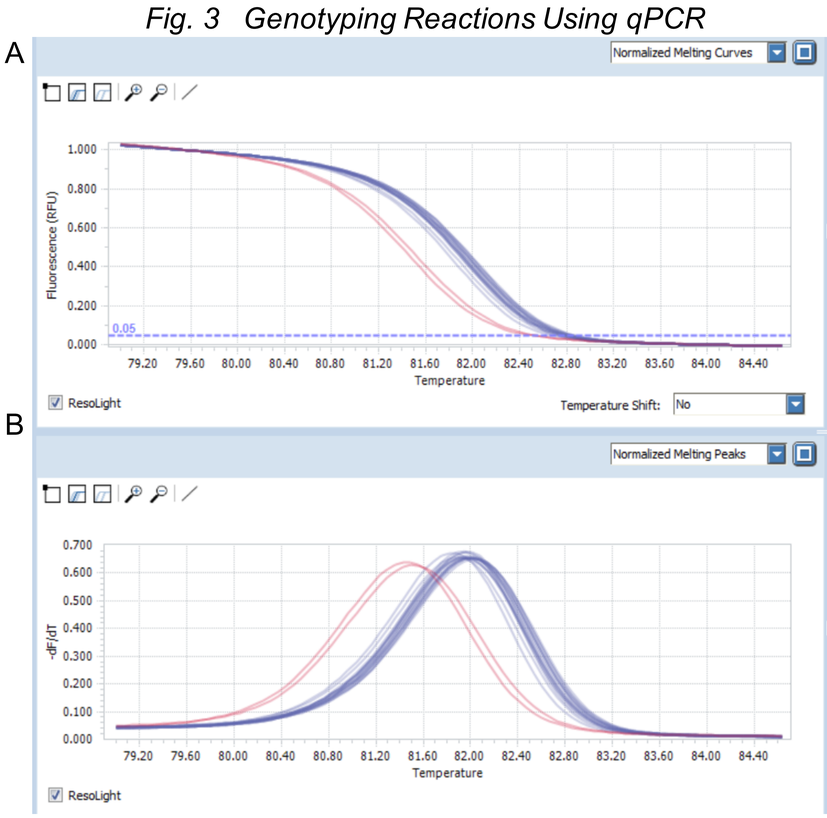


Current plot of the X chromosome. This plot shows the recombinant DNA that has been genotyped as a list on the right side and the green lines signify the position of the four primers currently being used. The red arrows are the P elements of the 2P-7 region.

qPCR reactions have been run at each of the four primer positions, 17.4Mb, 18.141Mb, 18.146Mb, and 18.24Mb, to genotype the DNA of interest.

The introgressed region between 17.4Mb and 18.141Mb shows conflicting results. Some of the DNA have been fertile while others have been sterile. This suggests that there is another possible sterility factor in the 2P-6 region flanking to the left.

Fig. 3 Genotyping Reactions Using qPCR



A. Normalized melting curves showing separation to distinctly determine *D. simulans* from *D. mauritiana.*

B. Normalized melting peaks which show the separation of peaks are found by calculating the derivative of the melting curves.

Reactions display graphs from the LightCycler machine reactions. The lines clustered around purple demonstrate DNA correlated with *D. simulans.* The two red lines signify *D. mauritiana* DNA, all reactions run in duplicate.

The separation in melting temperature is due to the nucleotide sequence differences between *D. simulans* and *D. mauritiana.*

CONCLUSIONS & FUTURE DIRECTIONS

Through the recombinant DNA produced up to this point we have narrowed the area of interest on the X chromosome from 300kb down to 100kb. By narrowing the region of interest we can now move toward looking for a gene of interest in this 100kb area. In this area there are many housekeeping genes, but the deletion of specific segments of the chromosome may lend to the sterile nature of the hybrid offspring.

We have decided to use two more primers in our region of interest in order to cut down the size of that region and narrow in on a specific region containing a gene or genes. We are also in the process of generating more recombinant flies to produce more DNA to further support the current findings.

ACKNOWLEDGEMENTS

1. Coyne, J. A. (1985). The genetic basis of Haldane's rule. *Nature, 314*(6013), 736-738. doi:10.1038/314736a0.
2. Orr, H. A. (1995). The Population Genetics of Speciation: The Evolution of Hybrid Incompatibilities. *Genetics, 139*(4), 1805-1813.
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4. Images using the University and UCARE logo were collected from http://ucare.unl.edu.

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