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What Are The Genes That Cause Male Sterility in Hybrid Offspring Between *Drosophila mauritiana* and *Drosophila simulans*?

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WHAT WE LEARNED

Finding genes that cause sterility in hybrid males between *D. mauritiana* and *D. simulans* will help us better understand evolution at the molecular level.

A genetic component related to sterility is near the 7.10 Mb region, but more research is needed to identify the gene or genes contributing to sterility

This will expand on the current understanding of speciation and shed light onto mechanisms of male sterility in insects that may help solve the challenges of insect disease vectors.

BACKGROUND

Research has found that the genes causing sterility in interspecific hybrids have a higher chance of residing on the X chromosome; this pattern is called the 'large X-effect'. Therefore this study focuses on the X chromosome and not the autosomes. The mechanisms underlying the large X-effect are not well understood. Previous research has demonstrated that the X-chromosome evolves faster than the autosomal chromosomes and regulatory phenomena such as spermatogenic X inactivation and dosage compensation can contribute to sterility within species, but further research is needed in order to better understand their effects on hybrid sterility. Researchers have used genetic approaches as well to studying species incompatibilities and have been successful. Other researchers have used different species of Drosophila and have discovered genes that may be responsible for the sterility in the male hybrid, but they have only discovered a few genes thus making this field an area of needed attention.

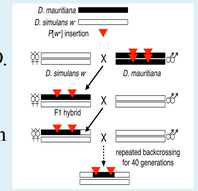
INTRODUCTION

The purpose of this research project is to better understand speciation - the process by which new species arise. To better understand how species form we will investigate how reproductive barriers come about-specifically the sterility of hybrid offspring will be the focus of this research. The species under investigation are *Drosophila mauritiana* and *D. simulans* that produce sterile hybrid males. These species are used specifically because they produce fertile hybrid females which allow genes to be moved between them. There are also published genome sequences for both species, which makes it is easier to identify candidate genes. These species are also easy to store, manage and cross to make hybrids. A genetic approach will be used in order to locate the genes responsible for male sterility. Specifically, we seek to identify genes from *D. mauritiana*, that cause male sterility when they are placed in *D. simulans*. This sterility results from hybrid problems in male reproductive tissues, specifically an inability to produce sperm. This research will focus on a specific region of the X-chromosome called 2P3, where two *P*-elements mark the *D. mauritiana* DNA that causes sterility.

METHODS

- Hybrid females were backcrossed to *D. simulans m*ales in order to move small chromosomal regions from one species to another
- Qualitative Polymerase Chain Reaction was used to determine if the DNA that was genotyped was either *D. mauritiana or D. simulans* at specific regions of the 2P3 X-chromosome.
- DNA Extraction was conducted to extract DNA from frozen hybrid flies that were previously generated.
- Gel Electrophoresis was used to separate and analyze the DNAs
- Data collection consisted of determining whether the DNA was *D. mauritiana* or *D. simulans* at the regions 6.20 Mb, 7.10 Mb, 8.02 Mb and 9.74 Mb. The average fertility scores for all the DNAs that consisted of both species were obtained for each region. Then a *t*-test was conducted in order to determine how significant the differences between the total average fertility scores were.

Fig. 1: Backcrossing between D. mauritiana and D. simulans. The region marked with the P[w+] marker is taken from one Drosophila species to another. The gene or genes can then be isolated for further experimentation.



RESULTS

Fig. 2 An example of Normalizing Melting Peaks and Amplification Curves. The melting peaks vary due to the different nucleotide sequences found in the D. simulans and D. mauritiana genome. The amplification curve displays the level of fluorescence following each PCR cycle.

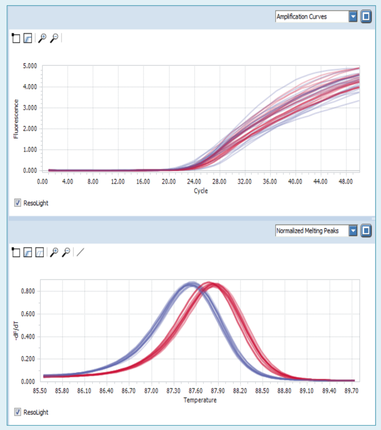
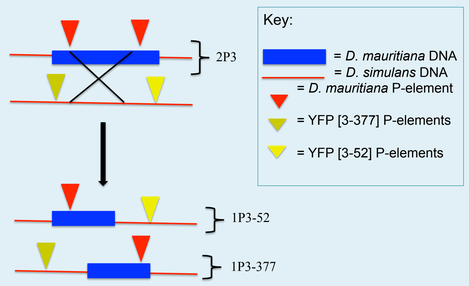
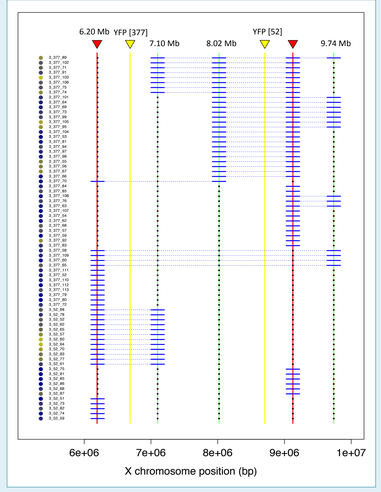


Fig. 3 Recombination event leading to 1P3-52 and 1P2-377 DNA



The 2P3 X-chromosome was recombined with a D. simulans X-chromosome in order to recombine the D. mauritiana DNA with D. simulans DNA with markers at the regions 377 and 52. The resulting DNAs were composed of one P[w+] and either regions 52 or 377 thus they were called 1P3-52 and 1P3-377. The resulting DNAs were then tested to determine where the D. mauritiana and D. simulans DNA were.

Fig. 5: Total average fertility scores for 1P3-377 and 1P3-52 DNAs at specific markers. Marker 1 represents region 6.20 Mb, marker 2 represents region 7.10 Mb, marker 3 represents region 8.02 Mb and marker 4 represents region 9.74 Mb. The total average fertility scores were obtained on a scale of 1 to 4 where 1 is the lowest, indicating flies that produced 0 offspring and 4 is the highest fertility because the flies produced 40 or more offspring.



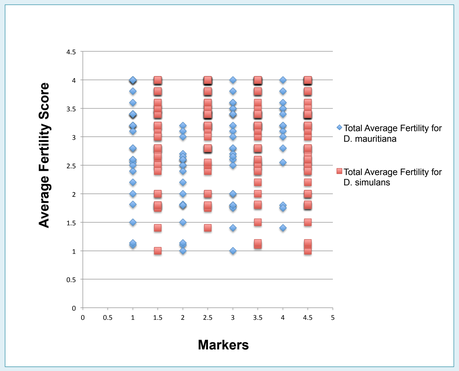


Fig. 6 Overview of all the hybrid DNAs genotyped with fertility scores, hybrid components and the primers used. The blue lines indicate D. mauritiana DNA and the black dots indicate D. simulans DNA. These were the genotypes that were found. The green lines are the regions that were under investigation. The yellow line lines represent the P-elements which are YFP [377] (left) and YFP [52] (right).

Conclusion

The data obtained starts with Figure 3, which explains how the hybrid male sterile flies were obtained. The 2P3 X-chromosome has dark orange eyes while the 1P3 X-chromosome has light orange eyes. A *t*-test was conducted on the fertility scores for DNAs with *D. mauritiana* and *D. simulans* alleles at each of the regions 6.20 Mb, 7.10 Mb, 8.02 Mb and 9.74 Mb. The *P*-value obtained for the regions 6.20 Mb was 0.85, for the region 8.02 Mb the *P*-value was 0.078 and for the region 9.74 Mb the *P*-value was 0.96. Since all of these values are >0.05, it means that the differences between the total average fertility score of both *D. mauritiana* and *D. simulans* were not significant at those regions. This means that the null hypothesis was accepted. The *P*-value for the region 7.10 Mb was 1.04E-06 which is below 0.05 therefore the null hypothesis can be rejected. Thus statistically there is a significant difference between the total average fertility score of genotypes that carry *D. mauritiana* and *D. simulans* alleles at the 7.10 Mb region on the 2P3 X chromosome. Figure 5 shows the difference between the fertility scores at all four markers. The graph shows that there is a difference in the fertility of the DNAs depending on whether they are *D. simulans* or *D. mauritiana* for 7.10 Mb region while the other regions show very little difference. Figure 6 shows that the 7.10 Mb region of the DNAs with the *D. mauritiana* DNA are very light in color, ranging from yellow to light blue indicating low fertility scores. This means that the gene or genes of interest might be very close to the 7.10 Mb region. The next step will be to design primers that increase the precision of estimates of the recombination break points to confirm and extend the results of this research.

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Citations

- Presgraves, D. C. (2008). Sex chromosomes and speciation in Drosophila. *Trends in Genetics, 24*(7), 336-343. doi:10.1016/j.tig.2008.04.007.
- Masly, J. P., & Presgraves, D. C. (2007). High-Resolution Genome-Wide Dissection of the Two Rules of Speciation in Drosophila. *PLoS Biology PLoS Biol, 5*(9), 243rd ser., 1890-1898. doi:10.1371/journal.pbio.0050243
- Ehime University. (2016). 愛媛大学. Retrieved April 11, 2016, from http://www.ehime-u.ac.jp/~scibio/evolut/DSEU_photo/mauritiana.html
- Strategic Communications and Planning (SCP). (2012). A Starting Point for Your Poster. Retrieved April 11, 2016, from http://posterbuzz.com/index.php/templates/
- Ehime - Strain Search Result -. (2002). Retrieved April 11, 2016, from https://kyotofly.kit.jp/cgi-bin/ehime/photo_data.cgi?SPID=260