Student in the Field: The Study of *Drosophila* Mate Preference as a Research Assistant in A. Moehring's Lab

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Background

My name is Joyce Liu and I am going into my 4th year of the Honours Double Major in Biology and Physiology program. I decided to pursue a double major because I was intrigued by many different aspects of the biological sciences. Specifically, I was very interested in translational research; how findings from basic science can be applied and used to further understand human diseases and behaviour. One area that I wished to learn more about was behavioural genetics. Through the Work Study program at Western, I was able to complement my studies with my interests by participating in research with Ryan Calhoun, a PhD candidate at Dr. Amanda Moehring's lab. I found this experience to be very rewarding because I witnessed first-hand the amount of dedication, hard work, and commitment that goes into research. It has really inspired me to push myself further in hopes of making a difference in the scientific community. In this article, I will further elaborate on the role that I played as a research assistant, how this experience changed my perception of research, and why it changed my future career goals.

Did you know that males of the genus *Drosophila* perform elaborate courtship displays such as tapping, singing and licking to convey visual, olfactory, gustatory, auditory, and tactile cues during courtship?¹ Female *Drosophila* then evaluate these signals when deciding to mate. Females of certain species of *Drosophila* also reject attempts made by males of other species to mate. The role of genetics on mate preference have always intrigued me, and one of the topics that I am interested in learning more about outside of the classroom. I am a fourth year Science student at Western pursuing an Honors Double Major in Biology and Physiology. As a Research Assistant at Dr. Amanda Moehring's lab, I worked under the supervision of Ph.D. candidate, Ryan Calhoun, to help locate genes of interest believed to account for mate preferences in *Drosophila*.

One key significance of *Drosophila* research is the fact that *Drosophila* share many homologous genes

with other species, including humans.² Many of these *Drosophila* genes play a role in the organism's behaviour, and scientists have demonstrated that these homologous genes are involved in a diverse array of behaviours such as learning, courtship, as well as cocaine tolerance and withdrawal.^{3,4,5} Studies have shown that the misexpression of certain *Drosophila* genes such as *sarah* (*sra*) in virgin female flies was associated with reduced receptivity to males.⁶ The same paper also stated that high levels of expression of DSCR1, the human homolog of *sra*, is associated with Down Syndrome⁷ and Alzheimer's Disease,⁸ although the role of DSCR1 in human mating behaviour is unclear. As stated by Pandey and Nichols (2011), "nearly 75% of human disease-causing genes are believed to have a functional homologue in the fly",⁹ thus *Drosophila* presents an excellent model organism for the study of human diseases and behaviour.

One emerging area of research is the potential for *Drosophila* studies to facilitate 'bench to bedside' translational research in understanding human behaviour and disease.¹⁰ *Drosophila* studies continue to provide great insight into this field by allowing scientists to make reliable, evidence-based predictions regarding the impact that mutation(s) of these homologous genes have on the welfare of humans. For example, researchers have found that a sensory neuron membrane protein (SNMP) is required for the reception of cVA, a pheromone in *Drosophila* that mediates sexual behaviour. SNMP has a human homologue, CD36, a scavenger receptor whose primary function is cholesterol and lipid uptake in vertebrates.¹¹ Although CD36 is known to be linked to a broad spectrum of disorders, its role in lipid transport and signal transduction is poorly understood; however, pheromone perception in *Drosophila* can be used as an excellent model to elucidate the role of CD36 in transmembrane signalling,¹¹ which may be involved in human behaviour.

Despite increased understanding in the role of genetics on behaviour, and the advancement of molecular techniques used to conduct these experiments to date, no naturally occurring gene has been identified to encode species-specific mating preference. It is important to note that the functions of many genes are pleiotropic: a single gene can contribute to the phenotype of several traits such that no single gene can account for all variation present within a specific phenotype, as is the case with mate preference.³

Dr. Amanda Moehring's lab is interested in identifying gene(s) that account for the interspecific discrimination exhibited by females of *Drosophila melanogaster* in mate preference. It has been demonstrated that *D. simulans* females reject copulation attempts from males of *D. melanogaster*. On the other hand, *D. melanogaster* females do not display this similar discrimination in mating behaviour and accept courtship and copulate with *D. simulans* males.¹² We are currently working to isolate potential mate preference loci within the tip of the right arm of the third chromosome which previous research has indicated is a region encoding female mate preference in *Drosophila*.¹³

In order to study the evolutionary neurobiology involved in *Drosophila* species divergence, multiple

species were studied in parallel: *D. melanogaster*, *D. simulans*, and *D. sechellia*. I ensured organizational protocols were diligently followed for the proper maintenance of the dozens of genetic lines under study, including those possessing deficiencies (*Df*), mutations (*Mut*), and balancer chromosomes (*Bal*). Deficiency lines contained flies that carried a deletion in a defined region of their genome¹⁴. This rendered *Df* fruit flies hemizygous at said region such that recessive alleles of *D. simulans* can be revealed and their phenotype can be observed in the F₁ hybrids when crossed with *D. melanogaster* females. If the deletion region contains genes that contribute to *D. simulans* female mate preference, these hybrids will display *D. simulans*-like discrimination against *D. melanogaster* males as opposed to hybrids that do not possess this deletion. *Mut* lines containing *P-element* insertions were also used to disrupted specific genes within particular regions of the chromosome under study. *Bal* are non-recombining chromosomes used to preserve genetic features on an opposing homologous chromosome for visible identification of genotype. Using the aforementioned tools we found several small regions of DNA in the female hybrids that lead to a *D. simulans*-like preference against *D. melanogaster* males. Furthermore, we used multiple *Mut* lines to look for gene(s) underlying interspecific discrimination in female *D. simulans*. In the future, we hope to support the finding that these small DNA regions contribute to the interspecific mate discrimination in *Drosophila* by conducting comparative studies in *D. sechellia*, a sister species of *D. simulans*.

To set up tests for interspecific female preference, I collected virgin females eight hours after eclosion, the emergence of the flies from their pupal casing. Then, I separated them under CO₂ anaesthesia, sorted them by visible phenotypic markers using a dissecting light microscope and transferred them to new standard 8-dram plastic vials containing approximately 7 mL of standard cornmeal and yeast medium. The *D. melanogaster* males were collected and housed using the same procedures. In addition, each cross vial was transferred into a new vial containing fresh medium every 5 days to ensure sufficient space and food for the growth and proliferation of the *Drosophila*. I also cleared the eclosing vials at regular time intervals in order to prevent *D. melanogaster* males' attempts to copulate and contaminate female virgins. Furthermore, I checked the

vials for potential mite contaminations using a dissecting light microscope to ensure the flies under study were not infested.

As a Biology student, studying behavioural genetics of *Drosophila* has greatly helped me solidify concepts learned inside the classroom. For example, concepts such as cAMP signaling pathways and the effects of pheromones on the perceived attractiveness of the opposite sex were discussed in my Physiology lectures¹⁵; literature has shown that learning and behaviour is dependent on the availability of catalytic subunits of PKA and its association with the cAMP signaling pathway. These concepts could potentially be related to behavioural questions surrounding mate preference that are being investigated exclusively from a genetics perspective in Dr. Moehring's lab. For me, being able to put theory into practice not only highlighted the significance of biological research, but also allowed me to develop a deeper appreciation for the commitment that researchers make in order to improve our understanding in the field of biology as a whole.

This experience has greatly helped me develop a sense of belonging in the broader scientific community, whether inside or outside the lab. Being a research assistant has allowed me to witness first-hand the amount of time and dedication that each researcher puts forth on a daily basis. I feel that each and every one of the researchers that I worked with really showed me the meaning of perseverance through his or her actions, and it is definitely something I admired most over the course of my time in the lab. It is these individuals' commitment to their work that has inspired me to pursue research, where I hope to make my contribution to the broader scientific community. Through future opportunities in my academic career, I will strive to conduct my own research, building upon our current findings to develop a deeper understanding of the significant role genetics has on the complex behaviours of not only the fruit fly, but also of human beings. I believe that studies of behavioural genetics in mate preference conducted in Dr. Moehring's laboratory could be a stepping stone in biological research. With more evidence from future studies to support our findings, the field of behaviour genetics may open up a new exciting area of research where we can investigate the intriguing topic of

predisposition of genes on human mate preferences through translational lessons in *Drosophila* studies.

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