MATING BEHAVIOR OF TWO POPULATIONS OF *Drosophila melanogaster*

By

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To my parents, family, and friends. Your love, support, encouragement, and belief in my potential helped me to grow and provided the support I needed to achieve my dreams.

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Drosophila melanogaster provides an unique opportunity to examine the important role of mating behavior in population divergence and isolation. Courtship of *D. melanogaster* is complex and involves many sensory modalities. The basic pattern consists of males orientating toward a potential mate and performing species-specific displays before attempting to copulate with the female (Greenspan and Ferveur, 2000). Candidate traits responsible for behavioral isolation in and among species have begun to be identified. Isolation due to differences in mating preference has been detected for several different populations of *D. melanogaster* (Korol et al., 2000; Hollocher et al., 1997; Haerty et al., 2002).

In this experiment multiple-choice and no-choice assays were performed with *D. melanogaster* to test for sexual isolation, mate discrimination for male courtship behavior, and evidence of differences in mating behavior in two geographically isolated populations wild-caught from Vancouver, B.C. and Leesburg, FL.

Similar to other sexual isolation studies, there was evidence of nonrandom mating between the two populations. Vancouver males obtained significantly more matings than Leesburg males when interacting with females of either population.

Female mate discrimination for male courtship behavior within each population could not be determined with any confidence. This may be partly because of the low number of assays that did not result in mating (thereby reducing the power to detect differences among copulating and non-copulating individuals): but could also be because of differences in traits that were not measured in this study such as pheromone profile or courtship song. However, as in other studies, male body size was found to be important in mating success, at least for the Leesburg males.

The goal of this study was to determine if there was evidence of sexual isolation due to differences in mating behavior between two outbred populations of *D. melanogaster*. Evidence of sexual isolation was not detected between the two populations (Vancouver and Leesburg). Some interesting mating patterns were uncovered and evidence of mate discrimination was found, but the behaviors responsible could not be determined.

INTRODUCTION

Sexual Selection and Speciation

Sexual selection is a form of natural selection, defined by Charles Darwin as "a struggle between individuals of one sex, generally the males, for the possession of the other sex"(Darwin, 1871). Darwin hypothesized two mechanisms underlying sexual selection: competition for mates and mate choice. Competition for mates is defined as any behavior within a sex that increases the number of potential mates for the winner. Mate choice is defined as the behaviors that reduce the number of mates by discriminating among the potential mates (Wiley & Poston, 1996). Either sex may use mate choice or compete for mates, but generally females are more discriminating, as females generally invest more in their offspring, and their fitness is not increased by the number of mates acquired but by the number of viable offspring produced. Conversely, males usually invest less and produce more gametes than females. Therefore, the number of mates acquired increases male fitness. Female mate choice occurs when the female uses certain male attributes or traits to discriminate one male over another. Females can therefore affect the fitness of males and the evolution of certain male attributes or traits (Andersson, 1994). Consequently, sexual selection can drive intrapopulation evolution, and through female choice can cause sexual isolation among populations, potentially leading to speciation.

Speciation is a complex process that may involve numerous mechanisms. Speciation occurs through the evolution of prezygotic and/or postzygotic barriers that

lead to reproductive isolation (Turelli et al., 2001). Speciation by sexual selection takes place when a change in female preference and a corresponding change in the sexually selected male traits in a population is the primary cause of prezygotic isolation and consequently reproductive isolation between populations (Kelly & Noor, 1996; Panhuis et al., 2001; Servedio, 2001; Kirkpatrick & Ravigne, 2002). It has been shown through population genetic models that changes in the way mates are acquired or chosen in a population can lead to rapid speciation because of the direct affects these changes have on gene flow (Lande, 1981;1982).

A pattern that suggests speciation by sexual selection is within-species variation in sexually selected traits and mate preference. For instance, incomplete reproductive isolation between populations could be the result of within-species, among population variation in sexually selected traits and mate preferences. Likewise, if the reproductive isolation between species is the result of differences in mating signals and mate preferences, and the species have little difference in other traits, then speciation via sexual selection is suspected (Panhuis et al., 2001). Therefore, it is important to understand whether sexual selection is playing a role in the difference between populations and/or species.

Drosophila as a Model Organism

The genus *Drosophila* is a good system to study sexual selection because of the elaborate courtship displayed by males toward females. Courtship within *Drosophila* is complex and involves many sensory modalities. The basic courtship pattern consists of males orienting toward a potential mate and performing species-specific displays before attempting to copulate with the female (Spieth, 1974; Spieth & Ringo, 1983). Courtship behavior composes most of the social behaviors observed and is an intrinsic feature of the

fly, in that a male fly raised in isolation is fully capable of performing all associated behaviors when presented with the correct stimuli (Greenspan & Ferveur, 2000).

Drosophila is also a valuable system for sexual isolation and speciation studies. Courtship behavior of related species groups in the genus are similar because of several behavioral elements of courtship originating in a non-sexual context (Spieth & Ringo, 1983); but within the order *Diptera*, courtship is widespread and diverse (Spieth, 1952). Many studies have already evaluated and described differences in related species of this genus that contribute to isolation and the possible mechanisms that contributed to isolation both behaviorally (Spieth, 1974; Spieth, 1952) and genetically (Greenspan & Ferveur, 2000; Sawamura & Tomaru, 2002).

Courtship Behavior of *Drosophila melanogaster*

In addition to classical genetic studies, aspects of courtship behavior are well studied in *Drosophila melanogaster* (Spieth, 1952; Bastock & Manning, 1955; Spieth, 1974). *Drosophila melanogaster* adults search for fermenting or decomposing plant matter as food sites, such as rotting fruits or flowers during a period in the morning and the late afternoon. In the course of time that the flies are at the food site, four major aspects of their life occur: feeding, courtship, mating, and oviposition. After mating, the females oviposit on suitable feeding substrates. When the offspring emerge from the pupal stage both sexes are immature. On emergence, the offspring disperse to secluded areas but return to neighboring food sites. Once they return to feeding areas they are exposed to stimuli of heterospecifics and mature conspecifics before becoming sexually mature. This allows immature individuals to learn to identify appropriate mates and receptive individuals (Spieth, 1974; Spieth & Ringo, 1983).

Initial studies of courtship behavior in *D. melanogaster* examined the complex series of behaviors that occur before copulation (Spieth, 1952; Bastock & Manning, 1955; Spieth, 1974; Markow & Hanson, 1981). As previously stated, courtship occurs at feeding sites where males indiscriminately approach feeding females. Before mating, the male first aligns with a potential mate and begins tapping the female with his front leg. After determining if the female is a potential mate, the male vibrates his wing toward her, producing a species-specific courtship song. He then circles around the female and contacts her genitalia using his proboscis ("licking"). After the genital licking, the male attempts copulation by bending his abdomen and thrusting his genitalia toward her. The male usually performs this series of behaviors repeatedly before copulation occurs (Greenspan & Ferveur, 2000). The female also plays an active role in courtship by performing a variety of acceptance and rejection behaviors. The acceptance behaviors are not especially overt and consist of slowing locomotor activity, preening, and genital spreading. Conversely, the rejection behaviors are more apparent and may terminate courtship. These behaviors are ovipositor extrusion; single or double wing flick; decamping (walking, flying, or jumping away); kicking; and abdominal elevation and depression (Spieth, 1952; Bastock & Manning, 1955; Spieth, 1974; Markow & Hanson, 1981). After the complex courtship behaviors, the pair may begin to copulate. Copulation lasts an average of 20 minutes, during which the male transfers seminal fluid into the reproductive tract of the female. The seminal fluid contains ejaculate composed of sperm and accessory gland proteins (Acps). The seminal fluid components have an effect on sperm storage, sperm transfer, and sperm competition in the female reproductive tract. The accessory gland proteins also have an effect on the female by

decreasing her receptivity, increasing ovulation rate, and affecting egg production (Wolfner, 2002). Additionally, the pheromone synthesis in females also changes, reducing her attractiveness and courtship elicitation from conspecifics. Post-mating changes that occur can last more than2 days (Ferveur, 1997).

The courtship behaviors of the male and female produce visual, olfactory, tactile, and auditory stimuli. Additional studies of courtship behavior sought to identify the numerous signals exchanged between interacting individuals. Markow (1987) found that different sensory stimuli are important for successful courtship in each sex. In males, visual stimuli from the female are needed to correctly perform courtship behaviors; females need auditory and olfactory stimuli to become receptive to a courting male.

The visual stimulus provided by the female is locomotion. The male must be able to stay in contact with the female and perform the correct behaviors; therefore, the male visual system is important. Additionally, an indicator of the female's receptivity and acceptance behavior is a decrease in locomotion, which the courting male must be able to perceive (Markow, 1987).

Auditory stimuli provided during courtship are also important for successful courtship. *Drosophila* males generate two kinds of acoustic signals during vibration of the wings: a pulse song and a sine song. These auditory signals consist of a species-specific pattern and presumably act in species recognition. The wing vibrations also function to stimulate females to become receptive (Greenspan & Ferveur, 2000) and lack of these acoustic signals results in a notable reduction of courtship success (Rybak et al., 2002).

Moreover, courtship provides olfactory stimuli that are important to both interacting individuals. Pheromones and their function in courtship were recently determined (Ferveur, 1997). Sex pheromones are chemical signals produced by both males and females and are presumably recognized in each sex by gustation during tapping and licking behaviors. Like the acoustic signals produced, pheromones or cuticular hydrocarbons function in species and mate recognition, and also in mate stimulation. For a given sex, strain, and age flies express a particular cuticular hydrocarbon pattern. For example, the cuticular hydrocarbons found on the female cuticle elicit precopulatory behaviors in males, and pheromones also enable males to discriminate potential from non-potential mates, i.e. virgin or receptive females vs. unreceptive females and males (Ferveur, 1997; Greenspan & Ferveur, 2000).

Initially it was assumed that *D. melanogaster* females mated indiscriminately, although after the work of Bateman (1948) and Petit and Ehrman (1969) sexual selection has been well established for this species.

Females of this species invest more in their offspring, and their fitness is not increased by the number of mates acquired but by the number of viable offspring produced. Conversely, males usually invest less and produce more gametes than females, therefore their fitness is increased by the number of mates acquired (Bateman, 1948). Because of the increased investment in gamete production, females produce limited number of ova (nutritionally demanding) and males produce excess sperm (low nutritional demands). Therefore, females are expected to be more discriminating (Spieth & Ringo, 1983; Andersson, 1994). Female choice is also expected to evolve when mating is costly, e.g. when mating causes an increase in the rate of predation and risk of

infection (Andersson, 1994). If females have the ability to choose their mates, then we expect female choice in this species because the act of mating reduces the female's fitness causing an increased cost to mating. The females suffer a reduction in reproductive success and longevity because of the male accessory gland products of the seminal fluid (Chapman, 2001; Wolfner, 2002). Therefore, the cost of mating makes female choice adaptive. Furthermore, females are essentially in control of whether or not they will mate with a particular male. They vigorously avoid undesirable copulations by performing a variety of rejection behaviors that usually terminates courtship (Bastock & Manning, 1955; Gromoko & Markow, 1993). An exception to this is newly emerged, teneral females who cannot perform rejection behaviors and consequently may be forced to mate (Markow, 2000).

Although females gain no direct benefits from mates, an increase in fitness associated with the opportunity for female choice was found (Promislow et al., 1998). This indicates a benefit for female choice and non-random mating. Additionally, the signals produced by the males during courtship may be energetically costly because courtship alone reduces longevity, therefore may provide information about their quality (Cordts & Partridge, 1996).

Now that sexual selection is known to occur in this species, many studies have begun to examine its role in this system. Most of the studies performed do not allow for analysis of sexual selection into its component parts, inter- and intra sexual selection (i.e. male-male competition and female choice) (Spieth & Ringo, 1983). However a number of studies have been able to look solely at the intersexual selection component. Female discrimination for males with certain phenotypes has been directly observed in many

studies (Bennet-Clark & Ewing, 1967;1969; Kyriacou & Hall, 1982; Partridge & Farquhar, 1983; Partridge et al., 1987; Scott, 1994; Wu et al., 1995; Bangham et al., 2002; Rybak et al., 2002). These studies identified potential traits used in female mate discrimination. These traits include body size, courtship song, and pheromone profile of the male. There is evidence that these traits influence the female's mating decision, and there may be an interaction between them. All of these traits may be related, for instance large males may be able to visually attract the female's attention, produce a louder courtship song, and produce higher quantities of pheromones than smaller males (Partridge et al., 1987; Rybak et al., 2002). Such studies have provided information on female responses to males of different phenotypes and some rules they use in mate discrimination, but more work is needed.

If all of the known potential traits are used in female mating decisions, which trait is more significant in her decision? Are there other unidentified traits that could influence her mating decision? The difficulty in pinpointing a single determinant of female mate discrimination (or male courtship success) is the result of factors that can influence sexual selection in nature are constantly changing, for example female mating status, age, size, and social environment, therefore a male trait that is a good predictor of courtship success in one environment may not be in another (Markow & Sawka, 1992).

Although the courtship behaviors were first described using an ethological approach (Bastock & Manning, 1955), most of the stimuli and their role in mate discrimination were discovered using sensory deficient mutants, transgenic lines, or surgical manipulation of sensory structures. Little attention has been given to the ethological approach of studying courtship, i.e. studying natural intra- and interspecific

variants or examining differences between natural populations. Little information is available for how much geographical and natural population variation exists in courtship behavior. Because of the natural social environment of flies differing greatly from the lab environment, results from most studies are not directly applicable to natural populations. Therefore, an important goal should be to try to understand natural population variation in the most natural setting possible to better understand the complexity and implications of sexual selection in this species.

Sexual Isolation and *D. melanogaster*

Courtship behavior differences can play a role within or between species in preventing gene flow (Butlin & Richie, 1994). There is great interest in analysis of geographic variation within species in courtship signals because this variation contributes to population divergence.

As previously stated, *Drosophila* is a good model for examining sexual isolation. Comparisons in traits related to courtship can be made within and between related species, because variation in courtship patterns between species usually differ in the relative frequency of each behavior but not in the overall pattern observed (Spieth, 1952; Bastock, 1956; Spieth, 1974). Furthermore, genetic dissection of species differences in courtship behaviors will aid in understanding how genetics and sexual selection are involved in reproductive isolation leading to speciation (Hollocher, 1998; Sawamura & Tomaru, 2002).

Many studies have already begun to gather evidence that factors affecting courtship are important in population divergence and isolation in this genus. Candidate traits responsible for reproductive isolation within and between species have been identified (Ewing, 1983; Coyne & Oyama, 1995; Savarit et al., 1999; Tomaru & Oguma, 2000;

Sawamura & Tomaru, 2002) . For example, interspecific differences in pheromones and courtship song may play a role in the sexual isolation between two sibling species, *D. melanogaster* and *D. simulans* (Sawamura & Tomaru, 2002).

Drosophila melanogaster provides a unique opportunity to examine the role of courtship related traits in sexual isolation. Until recently, it was assumed that *D. melanogaster* had a uniform, world wide range and exhibited no evidence of sexual isolation (Henderson & Lambert, 1982). However, three cases of sexual isolation between populations have been found. First, many studies have reported that Zimbabwe populations of *D. melanogaster* are sexually isolated from populations on other continents. Female mate discrimination and pheromone composition was found to be the dominant components in the divergence of Zimbabwe populations from all other populations (Wu et al., 1995; Hollocher et al., 1997; Takahashi et al., 2001). In another case, Korol et al. (2000) found that selection for stress tolerance resulted in behavioral divergence in female mate discrimination in populations of *D. melanogaster* on the slopes of "Evolution Canyon" producing sexual isolation between the populations. Lastly, Haerty et al (2002) found pre-mating isolation between two natural Congolese populations that may be because of a difference in pheromone composition.

Motivation

This study of sexual selection research was motivated because an understanding of the traits involved in sexual selection and reproductive isolation may provide insight into the forces that cause populations to diverge. Most approaches have been to manipulate a courtship signal (Greenspan & Ferveur, 2000) or summarize courtship behavior with the probabilities of transition between behaviors (Markow & Hanson, 1981). This provides information about the signals involved in sexual communication that differs between

species, but in order to determine the cause of sexual isolation, direct observation of individuals from one population interacting with individuals of another is necessary. Moreover, because most studies of courtship behavior in this species use sensory deficient mutants, transgenic lines, or surgical manipulation, little attention has been given to studying natural variation in courtship behavior.

Objectives

My approach involved two analyses with outbred populations of *D. melanogaster*, to determine if there is evidence of sexual isolation because of differences in mating behavior between two populations from different ecological environments. The populations were wild-caught from Vancouver, B.C. and Leesburg, FL. The study is laboratory based because using natural populations in the lab will allow me to control for: quality of the environment, age at testing, reproductive status, and population density during rearing. The questions I address is this research include

- **Question 1:** Do females of each population prefer to mate with males from their own population or with males of the other population?
- **Question 2:** What is the role of male courtship behavior and body size in female mate discrimination within each population?
- **Question 3:** What is the variation in female mate discrimination between populations?

No-choice (NC) assays were performed in a sex * population combination with four mating types possible (V * V; L * L; V * L; L * V; female and male respectively), as well as multiple-choice (MC) assays, to determine if females from each population prefer to mate with males from their own population. If the populations differ in mating behavior, I expect a decrease in mating success (increased copulation latency or no copulation) between individuals from the different populations in the NC assays.

Additionally, if the females prefer to mate with males from their own population, then I expect matings to deviate from random in the MC assays, with more females mating with males from their own population than the other population. However, in multiple choice experiments male-male competition cannot be excluded as the potential cause for mating patterns observed.

Previous research has suggested a possible role of body size, courtship song, and pheromone profile in mate discrimination for this species, but other traits involved in courtship have been overlooked. As stated previously, courtship in this species consists of elaborate and complex displays, and all of the behaviors involve visual, olfactory, tactile, and auditory stimuli. The behaviors involved in visual stimuli have not been thoroughly examined for a role in mate discrimination. These behaviors include chasing, orienting toward the female, licking, etc. In this study, I am interested in determining the relative importance of these behaviors, as well as body size in mate discrimination for each population. Males in the NC assays were characterized for the behaviors, and female discrimination for these male behaviors and/or body size was determined using copulation success and copulation latency as an indicator of female mate discrimination.

Using the data collected from the NC assays, I am able to determine the variation for males and females in the courtship related behaviors between the populations, as well as variation in female mate discrimination between the populations.

MATERIALS AND METHODS

Fly Populations

Vancouver Population (V)

The base population was collected (~50 mated females) in the summer 2001 in East Vancouver, B.C. The population maintained at 25°C, ~55% RH, on a 12:12 LD cycle. The larvae were raised at low density on a 14-day schedule. For more information contact Arne Mooers amooers@sfu.ca.

Forty mated females from the base population (>71,000) were used to start the Vancouver population used in the behavioral assays. The emerging offspring were mixed randomly for at least one generation in large two liter bottles with standard *Drosophila* medium before setting up at a constant density.

Leesburg Population (L)

The population was collected in the summer 2004 in Leesburg, FL. One hundred twenty mated females from the original population were used to start the Leesburg population used in the behavioral assays. The population maintained at 25°C, ~50% humidity, on a 12:12 LD cycle. The larvae were raised at low density on a 14-day schedule. The emerging offspring were mixed randomly for at least one generation in large two liter bottles with standard *Drosophila* medium before setting up at a constant density.

Rearing Conditions

After allowing for random mixing between all flies within a population they were setup on a two-week schedule. The populations were setup in 1/2 pint bottles to avoid crowding (Markow & Hanson, 1981). Sixteen bottles per population (8 originals and 8 backups) were setup at a constant density of 25 X 25 (females and males, respectively) with 50 mL of food (standard medium: cornmeal, yeast, molasses, tegosept, and proprionic acid). Flies were mixed among all replicate population bottles with each generation. Flies were allowed to mate for 5 days, then cleared to avoid larvae overcrowding. On Day 14, the setup was repeated.

Collection for Multiple-Choice Assays

Flies used in the MC assays were collected within 12 hours of eclosion under light CO_2 anesthesia. Collected individuals were kept until testing in vials containing ten individuals of the same sex. The food medium of each vial was colored red or green using one drop of food coloring per vial in order to identify the populations during the mating assays. Color was alternated for each population between assays to control for the effect of coloring on behavior (Som & Singh, 2002).

Experimental Setup for Multiple-Choice Assays

MC assays were performed between 0700 and 0900 hours, because of a lower circadian rhythm effect and high mating activity during this period (Sakai & Ishida, 2001). The mating assays were conducted in a temperature-controlled room. Multiple assays were performed each day simultaneously in 1/2-pint bottles with 5 mL of food in each. Males from each population were manually aspirated without anesthesia into the 1/2 pint bottle. Afterwards, females of each population were introduced, for at least 80 flies per mating bottle. The sex ratio was kept at 1:1, one male for every female for each

assay. Scan sampling was used to identify copulating individuals, which were aspirated out and identified based on sex and abdomen color (population source) using a microscope. Four mating types were possible: V * V, V * L, L * V, and L * L (female and male respectively). Assays were terminated before 50% of all possible matings had occurred to control for possible differences in mating propensity between the two populations (Casares et al., 1998), or for one hour, whichever occurred first.

Analysis of Multiple-Choice Assays

Contingency chi-square tests were performed for each replicate assay to test for deviation from random mating (Table 1). In order to test for deviations from random mating for all replicate assays, a $\sqrt{(\chi 2)}$ method was performed (Everitt, 1997; Panhuis et al., 2003). The square root of each $\chi 2$ was calculated for each assay; and its sign depends on the direction of the data. The sign direction was obtained by calculating the cross product of homotypic (V * V and L * L) minus heterotypic (V * L and L * V) matings from the contingency tables. The signed χ values have a normal distribution under the null hypothesis of random mating with a mean of zero and unit standard deviation. The standard normal variate Z was calculated for the MC assays (Table 1). Under the null hypothesis of random mating for all replicates, Z is the sum of all $\sqrt{(\chi 2)}$ values divided by $\sqrt{(n)}$, where n is the number of replicate assays. A calculated $Z \geq 1.96$ is significant at the 5% level.

Collection for No-Choice Behavior Assays

Flies used in the NC behavior assays were collected within 12 hours of eclosion under light CO_2 anesthesia. Ten males and ten females from each population were collected seven days before testing, by randomly choosing individuals from the

population bottles. The collected individuals were kept until testing in vials containing five individuals of the same sex. All individuals used in the assays were 7-day-old virgin flies. The benefits of using 7 day old individuals is that the discrimination ability and receptivity is increased compared to younger flies (Spieth, 1974; Manning, 1967).

Experimental Setup for No-Choice Behavior Assays

Each mating assay was digitally video recorded to allow for consistent behavior scoring. Assays were performed in a windowless, temperature controlled room and the mating chambers placed on a light table. By being lit from underneath small movements could be easily discerned, such as wing vibrations, preening, and licking.

A high resolution Sony DCR-HC85 MiniDV Handycam® Camcorder, with a focal length of 47 cm for recording was used. The camera was setup on a tripod 82 cm from lens to light box. The manual focus of the camera was set at 0.8 m and the manual exposure (open aperture) set at 10. This setup was repeated for all NC assays.

Assays were performed between 0800 and 1200 hours because of high mating activity and a decrease in the circadian rhythms effects during this time period (Sakai & Ishida, 2001). Assays were performed using mating chambers 35 mm * 10 mm high containing 1 mL of standard *Drosophila* medium. Each mating chamber was used only for a single assay.

For each assay, a single male and female were added simultaneously to a mating chamber by manual aspiration, immediately preceding the beginning of each assay. Video recording began after the introduction of the flies to the chamber and performed until copulation, or for 30 minutes as this is sufficient time for copulation to occur (Rybak et al., 2002; Manning 1967).

Both within population (V * V; L * L) and between population (V * L; L * V) assays were performed each day. The order of the assays was randomized each day to avoid an effect of day and time, which is known to influence behavior. A total of 40 assays for each mating type were performed. Assays in which the male failed to court the female were excluded from analysis resulting in approximately 37 hours of behavioral observations per sex.

Immediately after the assays, the male and female were measured for body size (μm). Measurements were made using an Olympus SZX9® dissecting microscope with a micrometer inserted into an eyepiece. Thorax length was measured, as this is a reliable estimate of body size for this species.

An observer who did not know the population source of the flies in the assays analyzed the videotapes. Focal animal sampling was performed to gather courtship behavior data for both the male and female using JWatcher™ 0.9 software (Altmann, 1974). This software was used as an event recorder that logs the time of each behavior when an assigned key is pressed. To characterize the males and females, behavior elements were defined for each sex that encompass *D. melanogaster* courtship (Speith, 1974).

For each assay, I recorded the time each individual was active, resting, or courting. When the male courted the female, 12 male and 8 female courtship behaviors were recorded. The following is a description of each courtship behavior recorded, along with an abbreviation for the behavior. Throughout the text, each behavior will be written using the abbreviation for brevity.

Male Courtship Behaviors:

- Tap (TAP): Male taps the female tarsus with foreleg
- Orienting toward the female during courtship:
    - Orient-back (OB): Orienting to the back of the female
    - Orient-front (OF): Orienting to the front of the female
- Licking: Male extends the proboscis to the female's genitalia while chasing (CHASE LICK) or orienting to the back (OB LICK) of the female
- Wing vibration of one or both wings by the male, based on position of the male to the female:
    - Chase + vibrate (CHASE WV): vibrates wings at a short distance from female
    - Orient back + vibrate (OB WV): vibrates when orienting toward the back of the female
    - Orient front + vibrate (OF WV): vibrates when orienting toward the front of the female
    - Attempted copulation + vibrate (AC WV): vibrates while attempting to mount the female
- Chasing (CHASE): male follows the female at a short distance
- Attempted copulation (AC): unsuccessful copulation attempt
- Copulation (COP): successful mounting of male on female

Female Courtship Behaviors:

- Wing flick (WF): Female flicks one or both wings
- Decamping (DECAMP): Female walks, flies, or jumps away from a courting male
- Kick (KICK): Female kicks courting male
- Ovipositor extrusion: Female extrudes ovipositor toward the head of the courting male while standing still (SS OVI) or decamping (DECAMP OVI)
- Abdominal elevation and depression (AED): Female elevates and depresses abdomen

- Preening (PREEN): Female preens while male is courting
- Stand still (SS): Female ceases locomotion

For all behaviors, I measured the frequency of occurrence or duration depending on whether the behavior is classified as an event (no duration) or a state (duration). By collecting data on frequency of occurrence or duration of each behavior, I was able to calculate:

- Proportion of time active, resting, or courting
- Copulation latency: period of time from the introduction of flies into the mating chamber to the onset of successful copulation
- Proportion of time allocated to performing each behavior during courtship: total duration of the behavior/total courtship duration
- Frequency of each behavior during courtship: number of times the behavior occurred/total number of behaviors performed during courtship

Analysis of No-Choice-Behavior Assays

Copulation Success

Chi-square contingency tests were performed to determine if there was a difference in the number of assays that resulted in copulation for V and L females and also for V and L males (Table 2).

Additional Chi-square contingency tests were performed to determine if there were differences in the number of assays that led to copulation for: V males with V females and L males with L females; V females with V and L males; L females with L and V males; V males with V and L females; L males with L and V females (Table 3).

Copulation Latency

A Kruskal-Wallis analysis of variance was performed to determine if copulation latency was different across mating types. Mann-Whitney U tests were also performed to

determine if there were differences in copulation latency observed for: V males with V females and L males with L females; V females with V and L males; L females with L and V males; V males with V and L females; L males with L and V females (Table 3).

Body Size

Mean body size (thorax length) of each sex was calculated for each population. A Mann-Whitney U test was performed to determine if male or female body size differs between the populations.

Body size of each sex was also calculated for copulating and non-copulating individuals of each mating type. Differences between non-copulating and copulating individuals in the mean body size for each sex was compared with Mann-Whitney U tests (Table 4).

Composite Measures of Behavior

The mean proportion of time each sex was active, resting, or courting (mutually exclusive categories) was calculated for copulating and non-copulating individuals of each mating type. Differences between non-copulating and copulating individuals in the proportion of time each sex was active, resting, or courting was compared with Mann-Whitney U tests (Table 5).

Courtship Behavior

Courtship behaviors that were observed in less than 10% of all assays were excluded from analysis. The behaviors excluded were OB LICK, SS OVI, DECAMP OVI, TAP, and KICK. These behaviors were difficult to observe, therefore inconsistent scoring of the observer could further complicate the low occurrence of these behaviors.

Additionally, for other behaviors many individuals did not exhibit the behavior, therefore the frequency distribution was skewed. These behaviors include: CHASE

LICK, OB, OB WV, OF, OF WV, AC WV, WF, and AED. Therefore, these behaviors were considered in analyses as a binomial response variable, and scored as either performing the behavior (1) or not performing the behavior (0).

The mean proportion of time allocated to, or frequency of each behavior during courtship, as well as the mean frequency of occurrence (0,1) was calculated for each mating type for copulating and non-copulating individuals (Table 6).

Before statistical analyses, the non-binomial courtship behavior data were either: square root transformed (frequency data), arcsine-square root transformed (proportion data), or log transformed (time data) to adjust for deviations from normality.

Discriminant Function Analysis

Discriminant function analyses were performed to determine whether copulating individuals could be discriminated from non-copulating individuals for each mating type based on male and female courtship behaviors and body size (Table 7). Both male and female behaviors and body size were included, since using only the behavior of one sex markedly reduced the percentage of cases correctly classified (results not presented).

Logistic Regression Analysis for Copulation Success

The effects of male and female courtship behaviors and body size on whether or not copulation occurred for each mating type were analyzed using logistic regression. Behaviors examined in the logistic regression were ones that had a standardized coefficient with an absolute value greater than 1 in the DF analysis. The standardized coefficients indicate the relative importance of each variable to the DF, however if none of the standardized coefficients were greater than $|1|$ then all of the behaviors were examined for an effect. The logistic regression was performed beginning with all

variables using backwards elimination to remove variables one at a time based on their significance to the model. Variables were eliminated if $P \geq 0.15$.

Multiple Regression Analysis for Copulation Latency

Although there were no differences in copulation latency across mating types (see Results), there may be a difference in the behaviors that are important for copulation latency for each mating type. Therefore, the effects of male and female courtship behaviors and body size on copulation latency for individuals that did copulate for each mating type were determined using multiple regression analyses. As in the logistic regression, the multiple regression was performed beginning with all variables using backwards elimination to remove variables one at a time based on their significance to the model. Variables were eliminated if $P \geq 0.15$.

All statistical procedures were performed using SPSS v. 12 (SPSS, 2003) or JMP IN v. 5.1 (SAS Institute, 2001).

RESULTS

Multiple-Choice Assays

Five of the replicate MC assays chi-square contingency tests resulted in rejection of the null hypothesis of random mating among individuals (Table 1). The $\sqrt{(\chi 2)}$ method performed to test for deviation from random mating for all replicate assays resulted in a Z value of 5.65, which led to rejection of the null hypothesis of random mating ($P < 0.001$; Table 1).

Overall, the number V * V copulating pairs accounted for 48% of all matings observed, whereas number of L * L copulating pairs only accounted for 16.5%. The number V * L copulating pairs accounted for 16%, similar to the percentage L * L copulating pairs, and interestingly the number of L * V copulating pairs accounted for a greater percentage of all matings than L * L with 19.5% of all matings observed.

V males achieved 67% of all copulations observed, whereas L males only achieved 33% of all copulations observed across all replicate assays.

No-Choice Behavior Assays

Copulation Success

There was no difference detected in the number of assays that led to copulation for V and L females ($P < 0.32$; Table 2); however, there was a difference in the number of assays that led to copulation between males of each population, with V males achieving copulation in more assays than L males ($\chi 2 = 5.68$, $P < 0.02$; Table 2).

A difference was found in the number of assays that led to copulation for V males with V females and L males with L females, with V males with V females having a greater number of assays that resulted in copulation ($\chi 2 = 5.48$, $P < 0.02$; Table 3).

There was no difference in the number of assays that led to copulation for V females with V and L males ($P < 0.20$; Table 3), however there was a difference in the number of assays that led to copulation for L females with L and V males, with the L * V mating combination more assays resulting in copulation when L females were interacting with V males ($\chi 2 = 4.24$, $P < 0.04$; Table 3).

Nevertheless, there was no difference in the number of assays that led to copulation for V males with V and L females ($P < 0.82$; Table 3). Similarly, there was no difference in the number of assays that led to copulation for L males with L and V females ($P < 0.28$; Table 3).

Copulation Latency

Copulation latency was not significantly different across mating types in the Kruskal-Wallis analysis of variance ($\chi 2 = 1.23$, $P < 0.75$). There was also no difference in copulation latency for: V males with V females and L males with L females ($P < 0.52$; Table 3); V females with V and L males ($P < 0.63$; Table 3); L females with L and V males ($P < 0.38$; Table 3); V males with V and L females ($P < 0.99$; Table 3); L males with L and V females ($P < 0.30$; Table 3).

Body Size

There was no difference between populations in female ($\chi 2 = 0.55$, $P < 0.46$) or male body size ($\chi 2 = 0.99$, $P < 0.32$).

Female body size was also not significantly different for non-copulating and copulating individuals of each mating type ($P \geq 0.05$; Table 4). Male body size was not significantly different for non-copulating and copulating individuals of the V * V and the L * V mating type ($P \geq 0.05$; Table 4). However, for the L * L mating type, copulating males were larger than non-copulating males ($\chi 2 = 6.49$, $P < 0.01$; Table 4). A similar trend was also observed for the V * L mating type, although the difference was not significant ($P \geq 0.05$; Table 4).

This suggests that body size was used by females when assessing males for the Leesburg population, but not for males of the Vancouver population.

Composite Measures of Behavior

There was no difference in the mean proportion of time active, resting, or courting of each sex for copulating and non-copulating individuals for the V * V mating type ($P \geq 0.05$; Table 5).

However, for the V * L mating type there was a difference in the proportion of time females were active ($\chi 2 = 9.66$, $P < 0.001$; Table 5) and resting ($\chi 2 = 6.15$, $P < 0.01$; Table 5) with non-copulating females spending a greater proportion of time active and resting than copulating females; copulating females were found to spend a greater proportion of time courting ($\chi 2 = 9.87$, $P < 0.001$; Table 5). Additionally, the males of this mating type exhibited the same pattern as females, with non-copulating males spending a greater proportion of time active ($\chi 2 = 10.53$, $P < 0.001$; Table 5) and resting ($\chi 2 = 7.34$, $P < 0.01$; Table 5) than copulating males and copulating males spending more time courting than non-copulating males ($\chi 2 = 10.31$, $P < 0.001$; Table 5).

For the L * V mating type, there was no evidence of differences in the proportion of time copulating and non-copulating females and males were active and courting, however non-copulating females and males were found to spend a greater proportion of time resting ($\chi 2 = 14.47$, $P < 0.001$ and $\chi 2 = 8.71$, $P < 0.001$ respectively; Table 5) than copulating females and males.

Comparisons for the L * L mating type between copulating and non-copulating males indicate no significant difference in the proportion of time males were active, resting, and courting, and there was no difference in the proportion of time females were active and courting ($P \geq 0.05$; Table 5), however non-copulating females were found to spend a greater proportion of time resting than copulating females ($\chi 2 = 4.82$, $P < 0.05$; Table 5).

These results suggest that males of both populations, when participating in unsuccessful courtship interactions with females from their own population have difficulty assessing the female interest; yet when interacting with a female of a different population, they tend to spend more time resting or active if the courtship interactions are unsuccessful. This pattern is also shown for V females. However, if the courtship interactions are unsuccessful, regardless of the interacting male's population, L females are more likely to spend time resting.

Discriminant Function Analysis

DF analyses performed to determine whether copulating cases could be discriminated from non-copulating cases for each mating type based on male and female courtship behaviors were able to correctly classify greater than 83% of non-copulating cases and greater than 83% of copulating cases for each mating type with an overall

percentage of greater than 85% cases correctly classified (Table 7). These results indicate that copulating and non-copulating cases can be distinguished based on male and female behavior for all mating types.

Logistic Regression Analysis for Copulation Success

In the V * V mating type assays, the only courtship behavior that predicted whether or not copulation would occur was whether or not the males exhibited OB (Logistic regression: OB (0,1): $\chi 2 = 5.68$, $P < 0.02$; Table 6). However, for all other mating types none of the measures for male and female courtship behaviors or body size predicted whether or not copulation would occur (L * L; V * L; L * V; Table 6).

However, there was a trend for non-copulating males to exhibit OB, OF WV, and AC WV more often than copulating individuals (Table 6). Moreover, a trend was observed for CHASE WV, with copulating males spending a greater proportion of time performing the behavior for all mating types except V * V, in which case the opposite trend was observed (Table 6). A trend was also observed for copulating females to exhibit the WF behavior more than non-copulating females for all mating types except V * V, in which case the opposite trend was observed (Table 6).

Multiple Regression Analysis for Copulation Latency

Three male behaviors, CHASE, OB (0,1), and AC, and one female behavior, PREEN, as well as female body size were significantly related to copulation latency for the V * V mating type (Multiple regression: r^2 adj = 0.39, $F_{6,16} = 3.37$, $P < 0.02$; CHASE: $\beta = -0.671$, $t = -2.24$, $P < 0.04$; OB (0,1): $\beta = 0.614$, $t = 3.14$, $P < 0.006$; AC: $\beta = -0.511$, $t = -2.67$, $P < 0.02$; PREEN: $\beta = 0.723$, $t = 3.73$, $P < 0.002$; FEMALE BS: $\beta = -0.61$, $t = -2.99$, $P < 0.009$). As the proportion of time allocated to CHASE and the frequency of AC

increased, copulation latency decreased. Similarly, as female body size increased, copulation latency decreased. However, as the proportion of time allocated to PREEN increased, copulation latency increased. Males that did not exhibit the OB (0,1) behavior were more likely to have a decreased time to copulation than males that exhibited the behavior.

Conversely, there was no evidence that male or female behaviors or body size was related to copulation latency for L * L mating type.

For the V * L mating type, only the female behavior WF (0,1) was significantly related to copulating latency (Multiple regression: r^2 adj = 0.183, $F_{1,16} = 4.820$, $P < 0.04$; WF (0,1): $\beta = 0.481$, $t = 2.20$, $P < 0.04$). Females that did not exhibit the WF behavior were more likely to have a decreased time to copulation than females that did exhibit the behavior.

For the L * V mating type, male body size along with the male behavior OF (0,1), as well as female behaviors, AED (0,1), PREEN, and SS, were significantly related to copulation latency (Multiple regression: r^2 adj = 0.422, $F_{6,16} = 3.67$, $P < 0.017$; MALE BS: $\beta = 0.788$, $t = 3.36$, $P < 0.004$; OF (0,1): $\beta = -0.579$, $t = -2.75$, $P < 0.014$; AED (0,1): $\beta = 0.465$, $t = 2.66$, $P < 0.017$; PREEN: $\beta = -0.836$, $t = -3.95$, $P < 0.001$; SS: $\beta = 0.563$, $t = 2.92$, $P < 0.01$). As male body size and the proportion of time allocated to SS increased, copulation latency increased. Conversely, as the proportion of time allocated to PREEN increased, copulation latency decreased. Males that did exhibit the OF behavior were more likely to have an increased time to copulation than males that did exhibit the behavior. Similarly, females that did not exhibit the AED behavior were more likely to have a decreased time to copulation than females that did exhibit the behavior.

Table 1: Copulation success for each mating type in the MC assays between the Vancouver and Leesburg populations (N = number of males and number of females from each population).

			Male				
Replicate	N	Female	V	L	$\chi 2$	χ	Z
1	80	V	42	11	0.08	– 0.28	5.65***
		L	14	3			
2	80	V	46	11	8.65**	2.94	
		L	11	12			
3	60	V	21	12	0.35	0.59	
		L	14	11			
4	80	V	34	16	0.10	– 0.32	
		L	20	8			
5	40	V	16	10	0.66	– 0.81	
		L	9	3			
6	80	V	39	16	0.38	0.62	
		L	16	9			
7	80	V	31	9	9.56**	3.09	
		L	18	23			
8	80	V	40	12	10.23**	3.20	
		L	12	17			
9	120	V	61	15	10.40***	3.22	
		L	23	21			
10	120	V	54	20	7.73**	2.78	
		L	22	24			

Assays were terminated before 50% of all possible matings had occurred. Results from the $\chi 2$ contingency tests determining deviation from random mating, $\sqrt{(\chi 2)}$ for each assay, and the Z value ($= \sum\chi/\sqrt{(n)}$). Significance level of: *P < 0.05, **P < 0.01, ***P < 0.001 indicates a significant departure from random mating.

Table 2: Copulation success of males and females from each population in the NC assays; results for the Chi-square contingency tests to determine if there was a difference in the number of assays that led to copulation for V and L females and for V and L males.

.		Copulated (N/Y)		
		N	Y	
Female	V	18	57	
	L	22	48	
				$\chi 2 = 1.0$ $P < 0.32$
		N	Y	
Male	V	14	60	
	L	26	45	
				$\chi 2 = 5.68$ $P < 0.02$*

*P < 0.05, **P < 0.01, ***P < 0.001

Table 3: Copulation success and copulation latency of interacting individuals of the within and between population assays in the NC assays; results for Mann-Whitney U tests and Chi-square contingency tests to determine if there were differences in copulation latency and in the number of assays that led to copulation; *P < 0.05, **P < 0.01, ***P < 0.001

Mating Type	Copulated (N/Y)	
	N	Y
V * V	7	32
L * L	15	20
Copulation Latency- $\chi 2 = 0.41$ P < 0.52		Copulation Success- $\chi 2 = 5.48$ P < 0.02*
	N	Y
V * V	7	32
V * L	11	25
Copulation Latency- $\chi 2 = 0.23$ P < 0.63		Copulation Success- $\chi 2 = 1.63$ P < 0.20
	N	Y
L * L	15	20
L * V	7	28
Copulation Latency- $\chi 2 = 0.77$ P < 0.38		Copulation Success- $\chi 2 = 4.24$ P < 0.04*
	N	Y
V * V	7	32
L * V	7	28
Copulation Latency- $\chi 2 = 0.00$ P < 0.99		Copulation Success- $\chi 2 = 0.05$ P < 0.82
	N	Y
L * L	15	20
V * L	11	25
Copulation Latency- $\chi 2 = 1.06$ P < 0.30		Copulation Success- $\chi 2 = 1.16$ P < 0.28

Table 4: Mean body size (± SE) for each mating type for copulating (COP) and non-copulating (NO COP) individuals for the NC assays, with differences compared with Mann-Whitney U tests.

	V * V			V * L			L * V			L * L		
Body Size	NO COP	COP	$\chi 2$	NO COP	COP	$\chi 2$	NO COP	COP	$\chi 2$	NO COP	COP	$\chi 2$
Male	40.83 (0.54)	40.96 (0.44)	0.03	38.90 (0.62)	40.35 (0.81)	2.84	41.00 (1.02)	41.08 (0.45)	0.01	39.92 (0.43)	42.00 (0.57)	6.49**
Female	48.86 (1.34)	46.92 (0.55)	2.51	46.20 (0.84)	47.39 (0.51)	1.37	45.50 (1.78)	47.44 (0.65)	2.15	46.43 (0.83)	46.13 (0.75)	0.04

N = 39, V * V; N = 36, V * L; N = 35, L * V; N = 35, L * L; *P < 0.05. **P < 0.01, ***P < 0.001.

Table 5: Composite measures of behavior of each sex for each mating type for copulating (COP) and non-copulating (NO COP) individuals in the NC assays; differences in the mean proportion (± SE) of time active, resting, and courting compared with Mann-Whitney U tests.

Proportion of Time	V * V			V * L			L * V			L * L		
	NO COP	COP	$\chi 2$	NO COP	COP	$\chi 2$	NO COP	COP	$\chi 2$	NO COP	COP	$\chi 2$
Female Active	0.34 (0.09)	0.36 (0.03)	0.26	0.51 (0.08)	0.23 (0.03)	9.66***	0.33 (0.08)	0.32 (0.03)	0.00	0.34 (0.04)	0.32 (0.03)	0.19
Female Resting	0.01 (0.00)	0.00 (0.00)	1.46	0.01 (0.00)	0.00 (0.00)	6.15**	0.07 (0.04)	0.01 (0.00)	14.47***	0.05 (0.02)	0.00 (0.00)	4.82*
Female Courting	0.66 (0.09)	0.64 (0.03)	0.11	0.47 (0.08)	0.77 (0.03)	9.87***	0.60 (0.09)	0.68 (0.03)	0.61	0.61 (0.04)	0.67 (0.04)	2.17
Male Active	0.32 (0.08)	0.36 (0.03)	0.34	0.51 (0.07)	0.23 (0.03)	10.53***	0.37 (0.08)	0.31 (0.03)	0.38	0.37 (0.04)	0.33 (0.03)	1.27
Male Resting	0.01 (0.01)	0.00 (0.00)	2.11	0.02 (0.00)	0.00 (0.00)	7.34**	0.02 (0.01)	0.00 (0.00)	8.71***	0.01 (0.00)	0.00 (0.00)	0.73
Male Courting	0.67 (0.08)	0.64 (0.03)	0.16	0.48 (0.08)	0.77 (0.03)	10.31***	0.61 (0.09)	0.69 (0.03)	0.29	0.62 (0.04)	0.66 (0.03)	1.27

N = 39, V * V; N = 36, V * L; N = 35, L * V; N = 35, L * L; *P < 0.05. **P < 0.01, ***P < 0.001.

Table 6: Courtship behavior measures for each mating type for copulating (COP) and non-copulating (NO COP) individuals in the NC assays; mean proportion of time allocated to performing each behavior during courtship (± SE), mean frequency of each behavior performed during courtship (± SE), or mean frequency of occurrence (0,1; ± SE).

	V * V		V * L		L * V		L * L	
Courtship Behaviors	NO COP	COP	NO COP	COP	NO COP	COP	NO COP	COP
Chase Proportion	0.14	0.16	0.27	0.14	0.22	0.17	0.23	0.18
	(0.04)	(0.03)	(0.05)	(0.03)	(0.10)	(0.02)	(0.03)	(0.03)
Chase WV Proportion	0.77	0.76	0.62	0.73	0.58	0.71	0.62	0.66
	(0.04)	(0.03)	(0.05)	(0.04)	(0.12)	(0.04)	(0.05)	(0.05)
Chase Lick Frequency (0,1)	0.86	0.59	0.73	0.76	0.86	0.64	0.87	0.80
	(0.14)	(0.09)	(0.14)	(0.09)	(0.14)	(0.09)	(0.09)	(0.09)
OB Frequency (0,1)	0.43	0.31	0.82	0.64	0.71	0.39	0.87	0.70
	(0.20)	(0.08)	(0.12)	(0.10)	(0.18)	(0.09)	(0.09)	(0.11)
OB WV Frequency (0,1)	0.57	0.59	0.45	0.40	0.57	0.46	0.20	0.60
	(0.20)	(0.09)	(0.16)	(0.10)	(0.20)	(0.10)	(0.11)	(0.11)
OF Frequency (0,1)	0.43	0.38	0.45	0.48	0.43	0.50	0.60	0.55
	(0.20)	(0.09)	(0.16)	(0.10)	(0.20)	(0.10)	(0.13)	(0.11)
OF WV Frequency (0,1)	0.86	0.56	0.73	0.52	0.43	0.64	0.73	0.65
	(0.14)	(0.09)	(0.14)	(0.10)	(0.20)	(0.09)	(0.12)	(0.11)
AC Frequency	0.09	0.07	0.11	0.13	0.11	0.09	0.10	0.11
	(0.03)	(0.01)	(0.03)	(0.02)	(0.02)	(0.01)	(0.02)	(0.02)
AC WV Frequency (0,1)	0.29	0.06	0.36	0.24	0.00	0.07	0.27	0.20
	(0.18)	(0.04)	(0.15)	(0.09)	(0.00)	0.05)	(0.12)	(0.09)
Decamp Proportion	0.80	0.82	0.85	0.76	0.78	0.74	0.79	0.74
	(0.05)	(0.03)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.05)
WF Frequency (0,1)	0.71	0.59	0.27	0.60	0.57	0.64	0.47	0.70
	(0.18)	(0.09)	(0.14)	(0.10)	(0.20)	(0.09)	(0.13)	(0.11)
AED Frequency (0,1)	0.14	0.38	0.27	0.36	0.14	0.39	0.20	0.30
	(0.14)	(0.09)	(0.14)	(0.10)	(0.14)	(0.09)	(0.11)	(0.11)
Preen Proportion	0.06	0.08	0.04	0.11	0.16	0.08	0.09	0.08
	(0.02)	(0.02)	(0.01)	(0.03)	(0.05)	(0.02)	(0.02)	(0.02)
SS Proportion	0.06	0.11	0.08	0.11	0.10	0.13	0.09	0.13
	(0.01)	(0.02)	(0.03)	(0.02)	(0.05)	(0.03)	(0.03)	(0.03)

Table 7: Discriminant function analyses to determine whether copulating cases can be discriminated from non- copulating cases for each mating type, with the percentage of cases correctly classified for non-copulating (NO COP) and copulating (COP) individuals, along with the overall percent of cases correctly classified for the NC assays.

Mating Type	NO COP Cases Correctly Classified (%)	COP Cases Correctly Classified (%)	Overall Correct Classification (%)
V * V	83.3	95.7	93.1
V * L	88.9	94.4	92.6
L * V	100.0	83.3	96.6
L * L	83.3	87.5	85.7

DISCUSSION

One objective of this study was to determine if females from each population prefer to mate with males from their own population, or with males of the other population. If the populations are sexually isolated or differ in courtship behavior, then females should mate more often with individuals from their own population, and there should be a decrease in mating success in interactions of individuals from different populations.

Results from the MC tests, indicate that V females prefer to mate with V males. Though, male-male competition cannot be excluded as a potential cause for the observed mating patterns, since in this setup males have the opportunity to interact. However, in the NC assays the percentage of NC assays that led to copulation for V females with V and L males was not different. Therefore, Vancouver females may in fact prefer to mate with males from their own population, however they are equally likely to mate with males from the other population when not given a choice.

The MC results, along with the observation that L males achieved copulation in fewer NC assays than V males, and that a greater percentage of assays led to copulation for L females with V males than with L males indicate that L females are more likely to mate with V males more than with males of their own population. Therefore, L females may prefer individuals from the Vancouver population.

Similar to other sexual isolation studies, there is evidence of non-random mating between the two populations (Wu et al., 1995; Korol et al., 2000; Haerty et al., 2002). However, the pattern seems to be driven by the high number of V * V matings. This may

be because of asymmetrical isolation for females of the Vancouver population, however additional experiments would need to be conducted to confirm this hypothesis.

Moreover, the increased copulation success of V males compared to L males for L females may be because of a novel male effect or the observed pattern could be because of L males being less “attractive” than V males, since L males obtained significantly fewer matings than V males in both the MC and NC tests.

Copulation latency was used in this study as an indicator of mating success, however for individuals that did copulate the time it took for these individuals to mate was not different across mating types, between the within population mating types, nor when interacting with individuals from the same or a different population. Therefore, copulation latency may not be a good indicator of mating success. For example, once females assess the male and decide either to copulate or not, the time it takes to mate may be related to a certain stimulus threshold required to successfully produce eggs or related to investment in egg production.

The second objective of this study was to determine the role of male courtship behaviors and body size, if any, in female mate discrimination within each population. Little information is known about the role these behaviors may play in female mate discrimination. Initially, it was assumed that visual cues were unimportant for mating success since *D. melanogaster* females could mate in the dark; yet it was found that mating success is significantly decreased when visual cues are lacking (Cobb & Ferveur, 1996). Furthermore, the lack of detailed studies may be partly because of the time required to make detailed observations of courtship interactions, increased interest in the role of pheromones and courtship song, and a negative publication bias.

In this study, when V males were interacting with V females, behaviors that were significantly or to some extent related to copulation success could not be identified; however there was evidence of a behavior that if performed indicated that copulation was unlikely to occur. Yet when V males were interacting with L females, copulation success was slightly correlated with the proportion of time allocated to CHASE WV. Similarly, when L males were interacting with L or V females, copulation success was slightly correlated with the proportion of time allocated to CHASE WV, and also correlated to male body size.

Similar to other studies, male body size was found to be important in mating success, at least for the Leesburg males (Partridge & Farquhar, 1983; Partridge et al., 1987). However, female mate discrimination for male courtship behavior within each population could not be determined with any confidence. This may be because of low number of assays that did not result in copulation, thereby reducing the power to detect differences among copulating and non-copulating individuals; but could also be because of differences in traits that were not measured in this study, such as pheromone profile or courtship song.

For assays in which copulation did occur, the proportion of time males spent in CHASE, the frequency of AC, and whether or not males exhibited OB was significantly related to copulation latency when V males were interacting with V females; however when interacting with L females, only body size and whether or not males exhibited OF was related to copulation latency. Conversely, there was no evidence that courtship behavior or body size was related to copulation latency when L males were interacting with L or V females.

The contradictions between behaviors identified as potentially important for whether or not copulation will occur and the behaviors related to copulation latency, may be because of once females assess the male and decide either to copulate or not, the time it takes to mate may be related to a certain stimulus threshold required to successfully produce eggs or related to investment in egg production.

The third objective of this study was to determine if there is variation between the populations in female mate discrimination. Since female discrimination for male courtship behaviors within each population could not be determined, it is difficult to determine the variation between the populations. The only consistent pattern observed was for body size, in which females of both populations interacting with L males mated more often with larger males.

Many studies have already begun to gather evidence that factors that affect courtship are important in population divergence and isolation in this genus. *Drosophila melanogaster* provides a unique opportunity to examine the role of courtship related traits in sexual isolation since three cases of prezygotic isolation between populations have been found (Wu et al., 1995; Hollocher et al., 1997; Takahashi, 2001; Korol et al.,. 2000; Haerty et al., 2002). The goal of this study was to determine if there is evidence of sexual isolation because of differences in mating behavior between two outbred populations of *D. melanogaster* from different ecological environments. Although, evidence of sexual isolation was not detected between the two populations, Vancouver and Leesburg, there were some interesting mating patterns uncovered and the exact mechanism driving these patterns remains to be elucidated.

Sexual selection can drive intrapopulation evolution, and through female choice or male-male competition can cause sexual isolation among populations, potentially leading to speciation. Population genetic models have shown that changes in the way mates are acquired or chosen in a population can lead to rapid speciation due to the direct effects these changes have on gene flow (Lande, 1981; 1982). Therefore, it is important to understand whether sexual selection is playing a role in the difference between populations and/or species.

Courtship behavior differences can play a role within or between species in preventing gene flow (Butlin & Richie, 1994). Therefore, there is great interest in analysis of geographic variation within species in courtship signals, because this variation contributes to population divergence. However, scarce information is available for how much geographical variation exists for *D. melanogaster*, as well as for many other species. Furthermore, genetic dissection of species differences in courtship behaviors will aid in understanding how genetics and sexual selection are involved in reproductive isolation leading to speciation (Hollocher, 1998; Sawamura & Tomaru, 2002).

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BIOGRAPHICAL SKETCH

Kelly Marie Jones was born on November 13, 1979, Fort Walton Beach, FL. She is one of three children of Marsha A. Jones and Wesley H. Jones. She followed her passion for biological sciences and enrolled at the University of Florida, where she earned her bachelor's degree in zoology. During her undergraduate education, she found an opportunity to perform research on the behavior of fruit flies, with Dr. Laura Higgins. After graduating, she enrolled in graduate courses at the University of Florida as a postbaccalaureate student. In 2003 she was accepted into the graduate program in the Department of Zoology at the University of Florida. She was awarded a Master of Science degree in May 2006.