I give permission for public access to my thesis and for copying to be done at the discretion of the archives' librarian and/or the College library.

_________________________________ _____________________

Jocelyn Packer May 2017

The Role of *desat1* in Courtship and Pheromone Synthesis of Male and Female *Drosophila Melanogaster*

By Jocelyn Packer

A Paper Presented to the

Faculty of Mount Holyoke College in

Partial Fulfillment of the Requirements

for the Degree of Bachelors of Arts with

Honor

Department of Biochemistry

South Hadley, MA 01075

May 2017

This paper was prepared

under the direction of

Professor Craig T. Woodard

for eight credits.

Dedication

To my wonderful family and friends who supported me throughout this project. I couldn’t have done this without you.

To my amazing flies whose privacy I repeatedly violated in the name of science.

Lastly, to Craig. Thank you so much for believing in me, especially when I didn’t believe in myself. You have patiently guided me, given me invaluable wisdom and your incredibly valuable time. I know that I could not have done this without you. Thank you so much!

Acknowledgements

I would like to first thank Craig for allowing me to be a part of your team. Everything I've done has only been possible with your help. You have been a mentored me with this project and academically but most importantly, you have mentored me in life. Every time I doubt myself, you are always there encouraging me. Throughout the week, I have many meetings that I dread going to but our weekly meetings were always the highlight of my week. Your unconditional support, amazing sense of humor and wisdom have guided me through these two year and I cannot thank you enough. I also would like to thank you for all the patience you had whenever I would struggle. You have allowed me to conduct research that I have truly developed a passion for and for that I thank you. I will miss you in the years to come. Also, I hope you can relax during your sabbatical. You definitely deserve it!

Secondly, I would like to thank the Biochemistry Department for allowing me to conduct this research.

I would also like to thank my amazing friends and family. I especially want to thank my mom for also being there and supporting. My mother never stopped believing in me and would often be on the phone with me while I was performing frustrating experiments. Thank you for being an amazing sounding board and a source of comfort and reassurance!

Next, I would like to thank my committee members Professor Amy Camp and Professor Kerstin Nordstrom for agreeing to read my thesis. I would also like to thank Professor Gary Snyder for tirelessly working with me and the GC-mass spec. I know that I often interrupted you when you were doing your own important work. I really appreciate all the time you gave me and all your amazing explanations.

I would also like to thank the Woodard Lab! You all are absolutely amazing! You all were there for me when I mumbling/yelling at my flies to mate. You all were also amazing soundboards for ideas on why the flies weren't cooperating. Without you all I genuinely do not think I would have been able to troubleshoot my way to results. I will definitely miss all the fun we had in lab. I would also like to thank Eliana Ramirez for performing the courtship assays with me. Your work was INCREDIBLY appreciated and I don't think I would have been able to do as much as I have without you.

Sincerely,
Jocelyn Packer

TABLE OF CONTENTS

INTRODUCTION....1

A. GENERAL OVERVIEW OF MAMMALIAN SEXUAL BEHAVIOR....1
B. SEXUAL MATURITY AND COURTSHIP BEHAVIOR....2
C. COURTSHIP PHEROMONES....7
D. GENES INVOLVED IN *DROSOPHILA COURTSHIP*....8
i. *fruitless*(*fru*)....*8*
ii. *doublesex* (*dsx*)....*11*
iii. *quick-to-court* (*qtc*)....*12*
iv. *desat1*....*13*

HYPOTHESIS....15

A. CREATING A MATING STANDARD....15
B. OBSERVING A DOUBLE MUTANT....15

MATERIALS AND METHODS....16

A. FLY STOCK CARE....16
B. VIRGIN COLLECTION AND AGING....16
C. FLY GENOTYPES....16
D. COURTSHIP ASSAYS....17
E. GAS CHROMATOGRAPHY-MASS SPECTROSCOPY....20
F. DOUBLE MUTANT MATING SCHEME....21

RESULTS....24

A. COURTSHIP ASSAY ANALYSIS....24
i. *Courtship Latency*....*24*
ii. *Courtship Index*....*26*
B. STATISTICAL ANALYSIS....27
C. CHROMATOGRAMS....27
i. *Male Chromatography Analysis*....*27*
ii. *Female Chromatography Analysis*....*29*
D. DOUBLE MUTANT....31

DISCUSSION AND FURTHER DIRECTIONS....32

A. COURTSHIP ASSAYS....32
B. CHROMATOGRAM....35
C. DOUBLE MUTANT....36
D. FUTURE DIRECTIONS....37

REFERENCES....39

List of Figures

FIGURE 1 4
FIGURE 2 5
FIGURE 3 6
FIGURE 4 10
FIGURE 5 11
FIGURE 6 12
FIGURE 7 17
FIGURE 8 22
FIGURE 9 25
FIGURE 10 26
FIGURE 11 30

Abstract

Sexual reproduction has fascinated scientists for centuries. The analysis of animal sexual behavior has helped to enlighten human sexual behavior (Ågmo and Ellingsen 2003). One area of contention in human sexual behavior is the effect pheromones have on humans. It has been argued that that due to our higher order of thinking, we can override the base biological need to choose a mate based on pheromones. However, through studying model organisms, such as *Drosophila melanogaster*, it can be seen that pheromones play a larger role in mate choice than originally thought (Pfaus et al 2003).

In this project, *Drosophila melanogaster* was used to study the role of pheromones in courtship. *Drosophila* males are known for an intricate courtship dance that is primarily controlled through the pheromones the males and females emit. However, there are several mutations that affect the pheromones and how they are interpreted by *Drosophila*. A mutation of interest is *desat1*. *desat1* is a mutation that affects male *Drosophila*. *desat1* mutants lack *desat1* desaturase. *desat1* desaturase promotes desaturation of the fatty acids that contribute to the courtship stimulatory pheromones. Without *desat1* desaturase, very few correct courtship stimulatory pheromones are produced (Ueyama et al 2005). Beyond *desat1* mutant's tendency to mate quickly, not much is understood about the effects the lack of courtship stimulatory pheromones have on other flies (Houot et al 2012).

There is also not much understood about a phenomenon observed in courtship assays between a *desat1* male, *desat1* female, and a wild-type female. If a *desat1* male is put in this situation, it will almost always choose the *desat1* female (Houot et al 2012). This phenomenon has not been widely studied and was the focus of the experiment. The literature states that the *desat1* females behave like wild-type females (Houot et al 2012). When *desat1* females are presented with wild-type males, their courtship assays are identical to that of the wild-type/wild-type pairings. However, no research has been done on whether or not there is a difference in the pheromones *desat1* females are emitting. It is possible that they are emitting a pheromone that, under normal conditions, has no effect on mating but when this pheromone is encountered by the *desat1* mutant, it overrides anything it senses from the wild-type female.

INTRODUCTION

A. General Overview of Mammalian Sexual Behavior

Understanding human sexual behavior has fascinated scientists for centuries. However, due to the sensitive nature of sex, and general societal views about sex, such studies have been made virtually impossible (Ågmo and Ellingsen 2003). This difficulty has shifted the study of human sexual behavior to animal models (Pfaus et al 2003). When it comes to mating, animals are said to be rational thinkers that make transitive choice (Arbuthnott et al 2017). Transitive choice, in the terms of choosing a mate, can be likened to deciding on an entree. You have three steps: sweet or salty, hot or cold, and then type of food. You would choose salty, hot and then you choose a steak. If you then see a sweet, hot, meat entree, you cannot choose that dish as the first and therefore most important criteria for the choosing an entree is salty. Therefore, you cannot choose this second entree as it doesn't meet the most important criteria, salty.

As the very basics of animal reproduction are essentially the same: arousal, initial mate selection, courtship, and copulation (Pfaus et al 2003). An animal's transitive choice will still help elucidate the mysteries of human mate selection (Arbuthnott et al 2017). From looking at animal models, such as rats, we have been able to better understand the hormone changes a woman experiences throughout her cycle and erectile dysfunction in men (Ågmo and Ellingsen 2003, Pfaus 1996). However, despite this success in using animal models to elucidate human behavior; it has been found that due to our greater mental capacity, biology and behavior do not always match. When analyzing human pheromones that are thought to influence courtship, it was found that the levels greatly varied between individuals (Mostafa et al 2011).

In addition to helping us better understand human sexual behavior, studying animal sexual behavior can help save certain species from extinction. As human expansion continues to encroach upon various animal habitats, the number of species on the endangered species list increases. Through understanding the minimum requirement certain species have for copulation to occur, can help us save many different animals. Also, because of the increased number of zoo and animal sanctuaries throughout the world, understanding animal mating behaviors has helped improve the habitats the animals live in and increase their populations in protected environments (Ågmo and Ellingsen 2003).

B. Sexual Maturity and Courtship Behavior

Through observing various animal species, we have increased our understanding of how we view our own method of choosing mates. The historical view of mate choice, human mate choice, is that a mate is chosen solely based on the attractiveness to the other individual (Ramanathan et al 2015). However, through observing bird mating habits, another layer of mate choice was found, mate choice copying. In various species of birds, females will want to mate with birds that have previously mated with other females. This led researchers to believe that human also choose mates based on mate choice copying. They tested the adage that a man is more attractive with a wedding ring (Vakirtzis et al 2010). They found that women were more critical of married men than unmarried men. However, they could find that it was not the symbol of marriage that made them more attractive but it was the woman they were paired with. When a man posed with an attractive “girlfriend” and without her, he was seen as more attractive when he was with her. This made them realize that the bird was choosing the previously mated males

because they found the female they previously mated with attractive. This study is a good example of how human sexual behavior can be understood using seemingly unrelated animals (Vakirtzis et al 2010).

As with most organisms, *Drosophila* must go through a growth cycle that starts from conception and concludes at the point of sexual maturity. Figure 1 illustrates a brief overview of the *Drosophila* life cycle. Sexual maturity is reached at the beginning of adulthood. Adulthood starts the moment after eclosion from the pupal case (emerging from the pupal case). The first week post-eclosion, are crucial for the final development of *Drosophila's* muscular and sensory systems. At the end of this week, *Drosophila* reach sexual maturity and can sense and respond to every courtship behavior they are presented (Blake et al 1995).

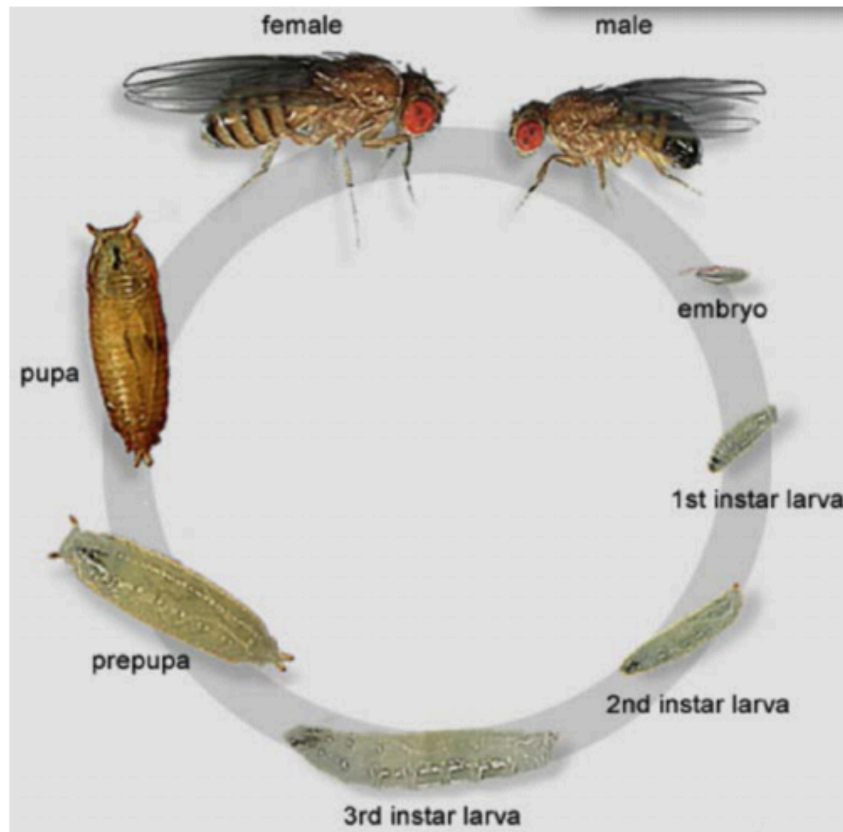


Figure 1. Life cycle of *Drosophila melanogaster*. *Drosophila* undergo an embryonic stage. Following the embryonic stage, the fly goes through three larval stages. The third instar larva wander and undergoes pupariation (the transition from larva to pupa). The third instar larva forms a pupal case. Within 10-15 minutes, the pupal case hardens and tans. At 12 hours after puparium formation, the prepupa transitions into the pupal stage. At the end of the pupal stage, the fly forms a pupal case and ecloses (emerges from the pupal case) as an adult fly. This process occurs over 9-10 days Retrieved from Weigmann et al (2003).

Drosophila mating is a very complex process that is genetically controlled (Enigma and Griffith 2007, Manning 1967). Successful courtship relies on the use of visual, chemical, tactile, and acoustic factors to complete the highly-specified mating dance. In *Drosophila melanogaster*, the male flies first initiate the dance by orienting. He primarily uses vision to find the female but in the dark, the male is able to find the female using her pheromones. He orients himself near the female and first taps her abdomen. This tapping allows him to perceive her personal pheromone

profile (Bontonou and Wicker-Thomas 2014). Orienting and tapping verifies that the other fly is truly female and healthy. If he finds her acceptable, he will extend the wing closest to her. That wing will begin to rapidly vibrate and will be the "love song" (Ferveur 2010).

The love song is a crucial part of *Drosophila* courtship. If a male is unable to produce this song, courtship will not occur (Rybak et al 2002). When a male has successfully located a female of interest, he will initiate the love song. He "sings" to her by extending his wing at an angle up to 90 degrees and rapidly vibrating it up and down. These vibrations are a means for the male to communicate that he is interested and fit for mating. This love song in addition to the pheromones emitted by the male is crucial for being considered a viable mate. The female perceives the song using her arista, which is found on the third segment of her antennae. The arista transmits this sound to the Johnston's organ's chordotonal sensilla in the antenna's second segment (Rybak et al 2002). There are two parts of the love song: a pulse song and a sine song as can be seen in figure 2 (Ewing 1983). The pulse song consists of a series of intermittent rapid clicks and the sine song consists of sinusoidal hum (Rybak et al 2002).

Figure 2. *Drosophila melanogaster* love song. An image of an oscilloscope recording of first the sine song, followed by the pulse song. Retrieved from Ewing (1983).

The production of vibrations during matings is not unique to *Drosophila*. Crickets also exhibit this behavior. However, the usage of the vibrations is slightly different. Crickets use the vibrations to communicate with all the females in his vicinity. A male cricket's vibrations are a

means to indicate that he is ready to mate (Libersat et al 1994). It is a form of wooing, but it is not targeted wooing as it is in *Drosophila*. In addition to vibrating their wings, *Drosophila* males also vibrates their abdomen. These vibrations are interpreted by the female and aid in her decision in allowing him to mate with her. The male will then orient himself behind the female and will use his proboscis to lick her genitals. Once this is done he will attempt to mount her by tucking in his abdomen and attaching himself (Bontonou and Wicker-Thomas 2014). Figure 3 illustrates the mating dance.

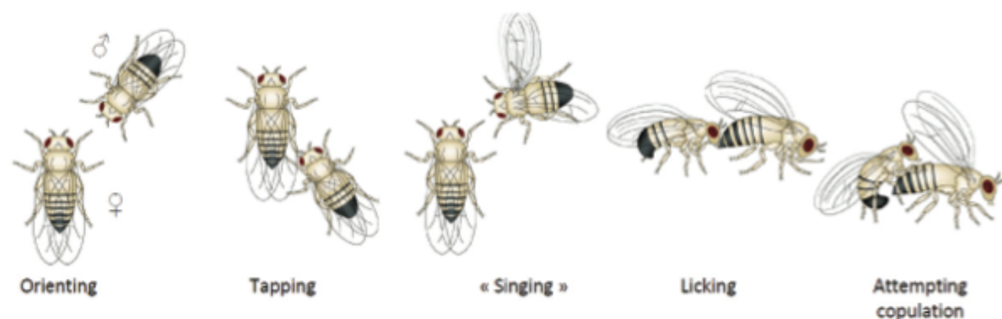


Figure 3. *Drosophila melanogaster's* courtship dance. Retrieved from Bontonou and Wicker-Thomas 2014.

At any point in this dance, the female can reject the male. Her rejections are typically through kicking the male away with her legs and running away to avoid copulation. The female will also move her abdomen up and down, close her wings to prevent copulation, or extrude her ovipositor, an organ used to lay eggs. The female shows acceptance of the dance be decreasing her movement, partially extruding her ovipositor, or releasing a droplet that excites the male (Bontonou and Wicker-Thomas 2014). It should also be noted that *Drosophila melanogaster* is the only species of *Drosophila* in which there are forced matings (Manning 1967). Matings that are considered forced are matings in which the female does not open her wings. After the male attaches itself to the female, she may be seen attempting to kick the male and attempting to

remove him. Typically, if the female is not successful in dislodging the male after 5 minutes. She will accept the mating (Manning 1967).

C. Courtship Pheromones

Pheromones are crucial to life among insects (Symonds and Wertheim 2005). They are essentially attractive and repulsive “scents” the flies emit to communicate with other flies. Pheromones are a special type of olfactory stimuli. They are created by the coenocytes and are long-chain cuticular hydrocarbons (CHCs) (Thistle et al 2012). Each gender, using their respective olfactory receptor, understands the same pheromone differently (Benton 2007). One of the most important, and the very first, task a male must complete in order to perform courtship is to identify females. In addition to sight, males will also use their antennae to find females. When a male is in close proximity to a female, the female’s pheromones will promote courtship behavior. In close proximity with a male, the other male’s pheromones will reduce courtship behavior in the male (Kallman et al 2015).

Females and males also emit different pheromones at different stages in their lives (Symonds and Wertheim 2005). Chemically, fly pheromones are long hydrocarbon chains that, as a minimum, must have a double bond in the Z7 (7-HC) position (Ueyama et al 2005). Pheromone biosynthesis happens as a result of unsaturated fatty acids being elongated to form very long chained fatty acids (Ueyama et al 2005, Thistle et al 2012). These long chains are converted to hydrocarbon chains that are one carbon shorter. This occurs by the *desat1* desaturase, desaturating fatty acid chains (Ueyama et al 2005).

The most powerful pheromone emission stage for females is in the first 18 hours of adulthood. Females produce the aphrodisiac pheromone, (Z,Z)- 7,11-heptacosadiene, in large quantities (Tompkins and McRoberts 1989). In females, this pheromone promotes arousal in males so that they will be encouraged to initiate the courtship dance (Tompkins and McRoberts 1989). In addition to this pheromone, female emit other pheromones that entices all males in the vicinity of the fly to come and attempt to mate. When other females sense these pheromone, in particular (Z,Z)- 7,11-heptacosadiene, they are warned to stay away from that particular female. When this pheromone is sensed by a female, it decreases her (Z,Z)- 7,11-heptacosadiene concentration and inhibits her desire to mate (Symonds and Wertheim 2005; Tompkins and McRoberts 1989). The same is true for males; they also emit pheromones that are enticing to females and repugnant to males (Symonds and Wertheim 2005). Males produce two male specific aphrodisiac pheromones, Z-II-octadecenyl acetate (cis-vaccenyl acetate) and Z-7-tricosene. These two pheromones make the female more receptive to mating (Tompkins and McRoberts 1989).

D. Genes Involved in *Drosophila* Courtship

i. *fruitless* (*fru*)

In wild type *Drosophila*, the male specific fruitless (*fru*) gene controls normal courtship behavior (Thistle et al 2012). *fruitless Drosophila* are mutants that are unable to successfully mate. The *fru* gene is a part of the gene sex determination hierarchy in *Drosophila*. *fru* is one of the genes control *Drosophila* morphology and sexual behavior. It also controls sex-specific

neural development (Swicki and Kravitz 2009). Due to the sex-specific nature of *fru*, it is primarily found in the neural cells of adult males. It was also found that *fru* males have altered olfactory receptor neurons in their antennae (Swicki and Kravitz 2009). There are a large number of dimorphic glomeruli on the antennae that had a higher volume than in the females. In addition to have a higher volume of the glomeruli, the *fru* males' individual glomeruli were much larger than in the wild-type.

This mutation primarily affects male courtship behavior. *fru* males can perform the courtship dance but they are unable to successfully latch on to the female's genitals. This is due to the muscles of Lawrence, found in the abdomen, are not properly formed (Gailey and Hall 1989; Gailey et al 1991). The muscles of Lawrence are found in the fifth segment of the male's abdomen (O'Dell and Kaiser 1997). These muscles are essential for the male to be able to tuck his abdomen in order to attach his genitalia to the female's (Gailey and Hall 1989; Gailey et al 1991). The mutant's muscles are shown in Figure 4.

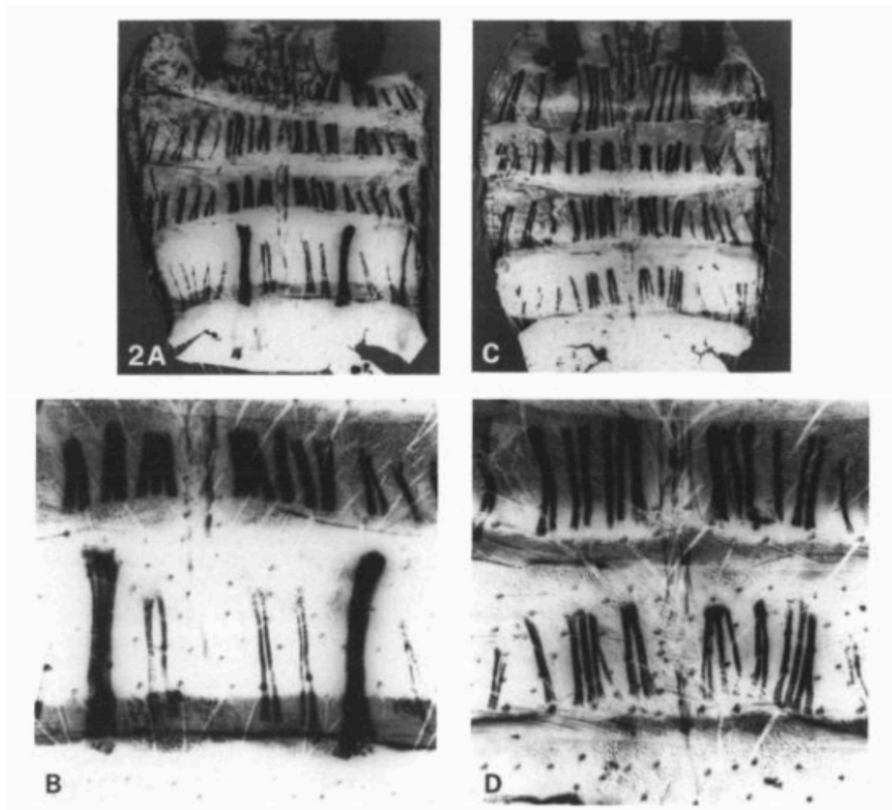


Figure 4. Wild type and *fru* mutant males' abdominal musculature. A and B represent the wild type abdomen. The two thick parallel lines that are magnified in image B are the muscles of Lawrence (MOL). In the mutants, C and D, the MOLs are not presents in the fifth segment as they are in the wild type (Gailey et al 1991).

As a result of the males being unable to successfully mate with females, they are sterile and have a low courtship index when presented with a female. As a result, the males will prefer courting other *fru*. When a group of *fru* males are put in a mating chamber, they will have *fru* courtship chain behavior. In *fru* courtship chain behavior, there will be a line of *fru* males following each other. They will, continuously extend and vibrate their wing without ever attempting to mount. The males are essentially courting each other without the apparent intention of mating copulating. The male that is being

courted typically does not show any behavior indicative of rejection (Gailey and Hall 1988). This is shown in Figure 5.

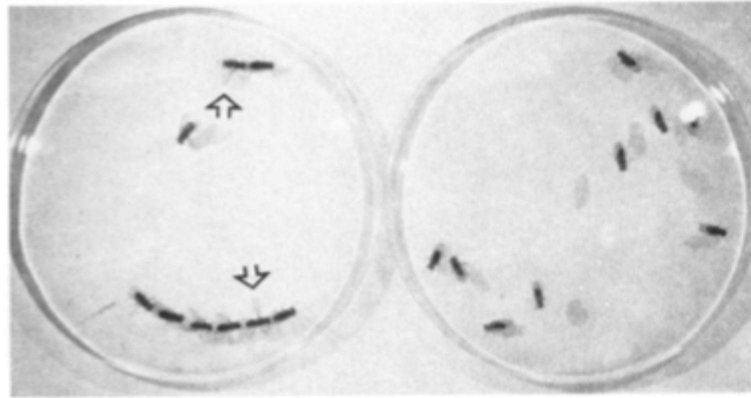


Figure 5. Image of *fru* mutant males in mating chambers. Both chambers only contain males. The chamber on the left contain *fru* mutants and the chamber on the right contain the wild type. The arrows present in the left mating chamber indicate that there are males whose wing has extended, thus indicating the courtship dance (Gailey and Hall 1988).

ii. *doublesex* (*dsx*)

The gene *doublesex* (*dsx*) controls normal development of the external sexually dimorphic characteristics (Kopp et al 2000). Essentially the isoform of *dsx* informs the organism of what sex it should be presenting as. *dsx* is one of the genes that controls the sex determination hierarchy in *Drosophila*. When *dsx* is present, through sex-specific alternative splicing, it encodes for protein isoforms that in both sexes (Rideout et al 2010). When *dsx* is not present, the repercussions of its absence is most easily seen in females. *dsx* females will have the same pigmentation pattern as males (Yamamoto et al 1997). This can be seen in figure 6. The
The females have similar markings as the males but they lack the male sex combs (Rideout et al 2010). Males can also be *dsx* mutants but the change in pigmentation is not as easily seen. In *dsx* mutants, proper formation of the genital discs are also affected in both genders. *dsx* males are

still able to successfully mate and the phenotypic changes are more acute than in the female (Emmons and Lipton 2003). *dsx* doesn't affect courtship behavior in either gender.

Figure 6. The image on the left shows the wild-type male's markings with an image of his sex combs. The image on the right shows a *dsx* female. Her markings are similar to the male but she lacks the sex combs. The male sex comb is circled on the left. (Rideout et al 2010).

iii. *quick-to-court* (*qtc*)

quick-to-court (*qtc*) is a mutation that affects male *Drosophila*. *qtc* is a gene that encodes a protein with a coiled-coiled domain (Houot et al 2012). In wild type flies the *qtc* gene is found in the olfactory organs, the brain, and the male reproductive tract. *qtc* controls normal courtship behavior and normal reception of female acceptance and rejection. *qtc* mutants have a higher prevalence of male-male mating than the wild type (Gaines et al 2000). When presented with females, *qtc* males will go through the courtship dance much faster than the wild-type males. This causes the females to have a higher prevalence of rejection and decreases the rate at which *qtc* males mate (Gaines et al 2000).

iv. desat1

desat1 mutants are no longer able to sense the repulsive pheromone from males and will attempt to mate with other males in addition to females (Houot et al 2012). This led scientists to discover that the cause of these abnormal mating patterns is not a mutation in the pheromone receptors of the males but a mutation in the pheromones the fly itself was emitting. *desat1* mutants lack *desat1* desaturase. *desat1* desaturase promotes desaturation of the fatty acids that contribute to the courtship stimulatory pheromones. *desat1* is normally localized and expressed in chromosome region 87C in both males and females (Labeur et al 2002). Without *desat1* desaturase, very few correct courtship stimulatory pheromones are produced (Ueyama et al 2005). Wild-type *Drosophila* males have pheromone profiles that are rich in monoenes, such as 7-tricosene (7T). Wild-type female *Drosophila* have pheromone profiles rich in dienes, such as 7,11-heptacosadiene (HD) (Labeur et al 2002).

Through rigorous analysis, researchers found that while very few courtship stimulatory pheromones were produced, *desat1* mutants do produce the wild-type pheromones in miniscule amounts. When these amounts are analyzed using chromatograms and mass spectra, water and typical components of air are found in more abundance that the normal aphrodisiac male pheromones (Houot et al 2012, Labeur et al 2002, Ueyama et al 2005).

Beyond *desat1* mutant's tendency to mate quickly, not much is understood about the effects the lack of courtship stimulatory pheromones have on other flies. There is also not much understood about a phenomenon observed in courtship assays between a *desat1* male, *desat1* female, and a wild-type female. If a *desat1* male is put in this situation, it will almost always choose the *desat1* female (Houot et al 2012). This phenomenon has not been widely studied and was one of the focuses of the experiment. The literature states that the *desat1* females behave

like wild-type females (Houot et al 2012). When *desat1* females are presented with wild-type males, their courtship assays are identical to that of the wild-type/wild-type pairings. However, no research has been done on whether or not there is a difference in the pheromones *desat1* females are emitting. It is possible that they are emitting a pheromone that, under normal conditions, has no effect on mating but when this pheromone is encountered by the *desat1* mutant, it overrides anything it senses from the wild-type female.

HYPOTHESIS

A. Creating a mating standard

In this experiment, the effects the *desat1* mutation has on fly mating habits were observed. It is known that *desat1* males will mate with flies of both genders. This mutation has only been found in male flies. Flies with this mutation slightly favor mating with males over females. However, when *desat1* male flies are presented with two females, and one is a wild type female and the other is a *desat1* female. The *desat1* males have shown a mating preference to the *desat1* females. Courtship assays were studied in order to verify that this is true.

B. Observing a double mutant

The *qtc*/*desat1* double mutant is known to have the same behavior as *desat1* males but they strongly prefer males. Very little research has been done on the double mutant male's courtship behaviors and the behavior of their females. Also, no research was done on the double's preferences toward the female. After breeding the mutant, these behaviors were analyzed in the hopes to create a standard for the double mutant's behavior and preferences.

MATERIALS AND METHODS

A. Fly Stock Care

Flies were stored at 25°C in bottles and vials. At the bottom of the bottles and vials was standard fly food made of Bloomington Nutri-fly with 10% Tegosept and 7.5% Propionic Acid.

B. Virgin Collecting and Aging

To collect virgins, several steps were taken to ensure that the collected flies were virgin. Bottles were taken and all the adult flies were removed. Cotton was placed on top of the food to ensure that the none of the adult flies remained in the bottle. The bottles were stored at 25°C for 8 hours to allow for normal development. All the newly eclosed flies were collected. To collect the virgin flies, they were all anaesthetized using CO_2. While anaesthetized, they were sexed based on sexual organs under a dissecting microscope. Once sexed, males and females were sorted and placed into separate vials. Both males and females were stored at 16°C (Tompkins and McRobert 1989).

C. Fly Genotypes

The control used to ensure normal courtship behavior is Canton-Special (CS). Both males and females were used in courtship assays to normalize both male and female mutant behavior. The *desat1* mutant flies and *qtc* mutant flies were bought from FlyBase.

D. Courtship Assays

Courtship assays were performed within 6 days of the flies being sexed. Using a mouth aspirator, males were placed first into a 0.2 cm^3 chamber (Figure 7). The males were given 3 to 5 minutes to acclimate to the chamber before the appropriate female was added. A maximum of three flies were added to the courtship chambers. Table 1 shows all the mating combinations observed.

FIGURE 7. Image of a mating chamber containing two males and one female.

Mating Assay Number	Number of Females Involved	Female Type	Number of Males Involved	Male Type
1	1	Wild-Type	1	Wild-Type
2	1	Desat1 homozygous	1	Desat1 homozygous
3	1	Wild-Type	1	Desat1 homozygous
4	2	1 Desat1 homozygous, 1 Wild-Type	1	Wild-Type
5	2	1 Desat1 homozygous, 1 Wild-Type	1	Desat1 homozygous
6	1	Wild-Type	2	1 Desat1 homozygous, 1 Wild-Type
7	1	Desat1 homozygous	2	1 Desat1 homozygous, 1 Wild-Type

8	1	Desat1 homozygous	1	Wild-Type
9	1	Desat1 heterozygous	1	Desat1 heterozygous
10	1	Desat1 heterozygous	1	Desat1 homozygous
11	0	-----	2	2 Wild-Type
12	0	------	2	2 Desat1 homozygous
13	0	-------	2	1 Desat1 homozygous, 1 Wild-Type
14	0	-------	2	1 Desat1 homozygous, 1 heterozygous

Table 1. Table illustrating the courtship assays performed. CS flies were used as my controls.

Courtship behavior was observed using iOS 10. Each mating of interest was observed for 30 minutes or until copulation occurred. The courtship latency (CL) of each mating, or the

indicator of successful copulation, was determined to be high, medium, or low. High CL was determined to be the occurrence of copulation within 10 minutes. Medium CL was the occurrence of copulation between 10 and 30 minutes and low CL indicated that copulation did not occur within 30 minutes. Each CL of high, medium, and low was given a score of 3,2, and 1 respectively. Components of the protocol were taken from Gaines et al 2000.

The courtship index (CI) of the matings was also observed where CI represents whether courtship behavior was observed and the extent to which the males exhibited courtship behavior. Components of the protocol were taken from Gaines et al 2000. The behaviors determined to be courtship behavior were orienting, shaking the abdomen, tapping, wing vibration, licking, mounting, and copulation. These behaviors were ranked in the order of occurrence where orienting, shaking the abdomen, and tapping are considered low CI's. All the previous behavior in addition to wing vibration and licking were medium CI's. High CI was determined to all the courtship behavior up to and including copulation. Each CI of high, medium, and low was given a score of 2, 1, and 0 respectively.

E. Gas Chromatography-mass spectroscopy (GC-mass spec)

To analyze the pheromones of the flies, using the protocols in Tompkins and McRobert 1989 and Gaines et al 2000's paper, a gas chromatography/ mass spectroscopy (GC mass spec) was performed to analyze the pheromones. An HP-5MS (Crosslinked 5% PH Me Siloxane) was used. To prepare the samples, flies were sorted and placed in vials the night before. The day of sample preparation, flies were cold anaesthetized. To cold anaesthetize the flies, a vial containing the flies of interest were completely immersed in an ice bucket. They stayed completely immersed in the ice for 5 minutes or until there was no detectable movement. The vials were

then partially pulled out of the ice buckets and the flies were removed using a brush. The flies were placed in a 100 µL solution of HPLC-grade n-hexane. A minimum of three flies and a maximum of five flies were placed in the solution. The flies soaked in the solution for 5 minutes with sporadic gentle agitation. At the end of the five minutes, the flies were vortexed for 1 minute and the flies were removed. The cuticular extracts were stored at 4°C in 1mL glass vials with Teflon lined caps. The cuticular extracts were stored for 12 hours and at the end of the 12 hours, they were analyzed. After 12 hours, 5µL of 100µL sample was injected into the GC-mass spec column. The column's temperature was increased from 150°C to 300°C at a rate of 5°C/min. Eicosane was used as the internal standard. This was procedure was performed with both wild-type and *desat1* flies of both genders.

F. Double Mutant

The double mutant was first bred from the 12520 *desat1* mutant and the 40242 *qtc* mutant retrieved from FlyBase. These two mutants were chosen because of their phenotypes. The *desat1* mutant has normal eyes, which are colored eyes, and a normal body. The *qtc* mutant has yellow colored bodies and white eyes. The desired double mutant will never have white eyes or yellow bodies and its phenotype matches the *desat1* homozygous mutant's phenotype. The mating scheme can be seen in figure 8. In figure 8, not all of the F4 generation will have be double mutants. The double mutants and non-double mutant flies will be phenotypically similar. To remedy this, single pair matings were performed. After the observation of approximately 5 generations, if the resulting progeny never have white eyes and/or yellow bodies, those flies were then deemed to be the double mutants. Those double mutant flies were then used to build a stock of double mutants for testing.

Color is used to trace particular genotypes.

Male **Female**

P

$\frac{+\ \mathrm{w}}{----};\frac{+}{+};\frac{desat1,\mathrm{w}+}{desat1,\mathrm{w}+}$ **X** $\frac{\mathrm{yw}}{\mathrm{yw}};\frac{qtc,\mathrm{y}+}{qtc,\mathrm{y}+};\frac{+}{+}$

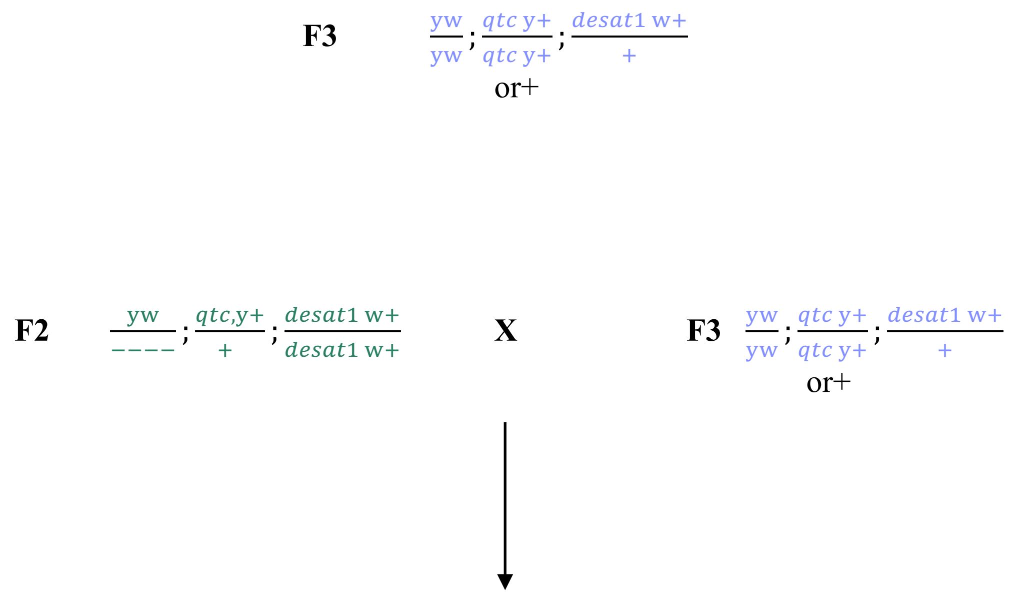
F1 $\frac{\mathrm{yw}}{----};\frac{qtc,\mathrm{y}+}{+};\frac{desat1,\mathrm{w}+}{+}$

Male **Female**

F1 $\frac{\mathrm{yw}}{----};\frac{qtc,\mathrm{y}+}{+};\frac{desat1,\mathrm{w}+}{+}$ **X** $\frac{+\ \mathrm{w}}{----};\frac{+}{+};\frac{desat1,\mathrm{w}+}{desat1,\mathrm{w}+}$

F2 $\frac{\mathrm{yw}}{----};\frac{qtc,\mathrm{y}+}{+};\frac{desat1\ \mathrm{w}+}{desat1\ \mathrm{w}+}$

F2 $\frac{\mathrm{yw}}{----};\frac{qtc,\mathrm{y}+}{+};\frac{desat1\ \mathrm{w}+}{desat1\ \mathrm{w}+}$ **X** $\frac{\mathrm{yw}}{\mathrm{yw}};\frac{qtc,\mathrm{y}+}{qtc,\mathrm{y}+};\frac{+}{+}$



Now some of the F3 progeny will be the double mutant

F4

$$\frac{yw}{yw};\frac{qtc,y+}{qtc,y+};\frac{desat1,w+}{desat1,w+}$$

or+   or+

Set up, in 20 individual vials, 20 separate matings between single F4 males and single F4 females (20 single pair matings).

Examine each line afterward for multiple generations. The lines that NEVER have any white eyes or yellow bodies, must be genotype:

$$\frac{yw}{yw};\frac{qtc,y+}{qtc,y+};\frac{desat1,w+}{desat1,w+}$$

Figure 8. Description of the mating crosses to get the *qtc/desat1* double mutant. The figure is color coded as some mutant genotypes are used more than once.

RESULTS

A. Courtship Assay Analysis

i. Courtship Latency (CL)

To analyze the courtship latency (CL), each trial was scored and given a number of either 1, 2, or 3 based on the mating times. A score of 1 indicates a low CL, meaning that copulation did not occur within 30 minutes. A score of 2 indicates a medium CL indicates that courtship occurred between 10 and 30 minutes. A high CL was given a score of 3 and represents the occurrence of copulation within the first 10 minutes. Figure 9 shows the CL of the males with respect to the individual or individuals they were paired with. There was no difference between the CL's of males presented with *desat1* females and wild-type females.

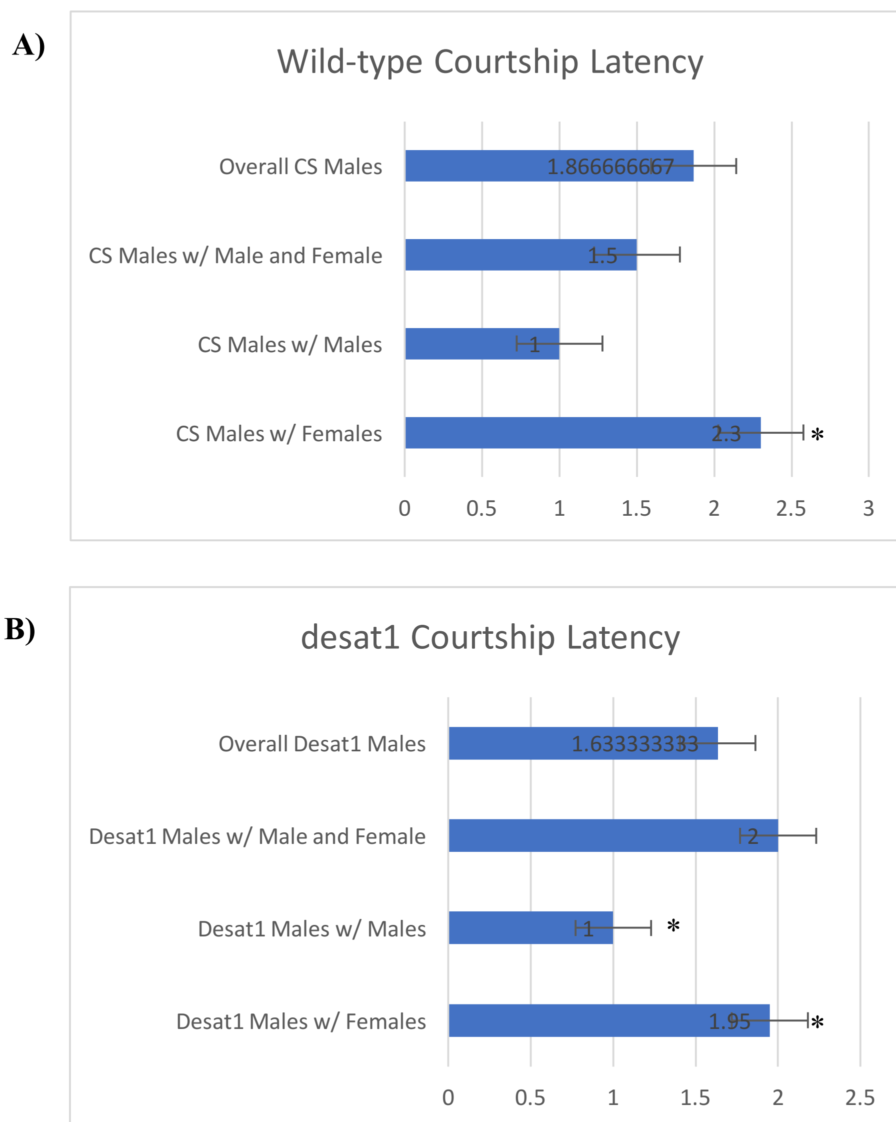


Figure 9. The average CL of wild-type and *desat1* males. A) The average CL of wild-type (CS) males. The overall CL of the CS, wild-type, males were 1.867. In the trials where the wild-type was presented with both males and females, the CL was 1.5 and the CL of wild-type males presented with other males was 1. When presented with only a female, the CL was 2.3. B) The average CL of *desat1* males. The overall CL of the CS, wild-type, males were 1.633. In the trials where the *desat1* males were presented with both males and females, the CL was 2 and the CL of *desat1* males presented with other males was 1. When presented with only a female, the CL was 1.95. There is an asterisk next to the values that are statistically significant.

ii. Courtship Index

To analyze the courtship index (CI), each group of behavior was given a score of either 0, 1, or 2. A score of 0 represents orienting, shaking the abdomen, and tapping. Wing vibration and licking was given a score of 1. A score of 2 represents the occurrence of copulation. Figure 10 represents the CI of the males with respect to the individual or individuals they were paired with. There was no difference between the CI's of males presented with *desat1* females and wild-type females.

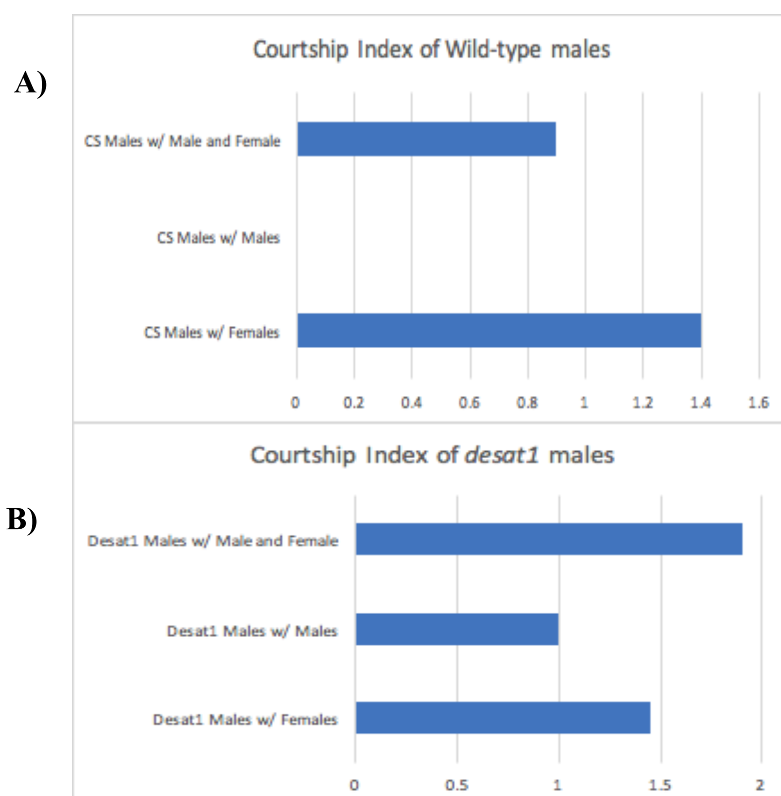


Figure 10. The average CI of wild-type and *desat1* males. A) The average CI of wild-type (CS) males. The CI of the CS, wild-type, males in trials where they were presented with both male and females was 0.9. The CI of the CS, wild-type, males in trials where they were presented with males was 0. The CI of the CS, wild-type, males in trials where they were presented with females was 1.4. B) The average CI of *desat1* males. The CI of the *desat1* males in trials where they were presented with both male and females was 1.9. The CI of the *desat1* males in trials where they were presented with males was 1. The CI of the *desat1* males in trials where they were presented with females was 1.45.

i. Statistical Analysis

An ANOVA test was performed to analyze the differences in courtship latency (CL). This test was employed for comparing the CL of the CS and *desat1* males. Using the ANOVA test, it was found that there is a statistically significant difference between the groups. The F value was 5.06 and the F_{crit} was 3.22. As the F value, 5.06, is greater than the F_{crit} value, 3.22, the null hypothesis can be rejected and my results are significant. A post hoc ANOVA test, Games Howell, was then used. It found that the CS male with females, *desat1* male with males, and the *desat1* male with females were significant. This is illustrated with an asterisk in figure 9.

C. Chromatograms

i. Male Chromatograms

Eicosane served as a standard for the point at which peaks start to be analyzed. Eicosane had a retention time of 12.60 meaning that only peaks succeeding retention times of 12.60 were eligible for analysis to be considered a possible aphrodisiac pheromone. Figure 11 shows the chromatograms of the CS and *desat1* males respectively. The CS chromatogram had the aphrodisiac pheromone, Z-II-octadecenyl acetate (cis-vaccenyl acetate), to have a retention time of 17.41. This peak can be seen in figure 11A. In figure 11B, the *desat1* males also had the Z-II-octadecenyl acetate (cis-vaccenyl acetate) peak with a retention time of 17.41. The mutants had this in a greater abundance than the CS males. The CS males' peaks corresponding to the aphrodisiac pheromone Z-7-tricosene with a retention time of 13.07. The *desat1* males did not

have a noticeable peak corresponding to Z-7-tricosene with a retention time of 13.07. These peaks were considered to correspond with these pheromones by comparing their mass spectra to the values determined by the National Institute of Standard and Technology (NIST).

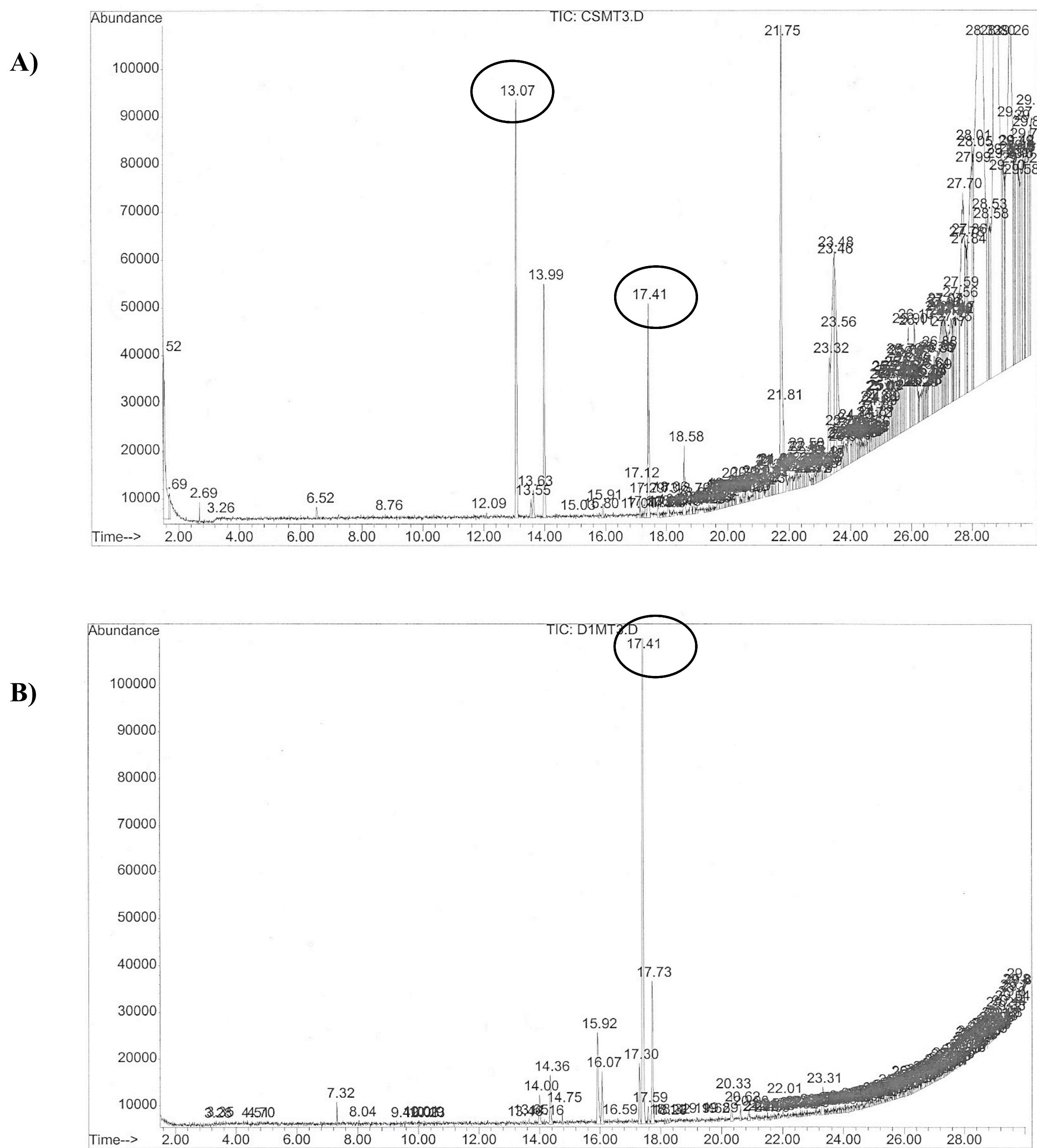


Figure 11. CS and *desat1* male chromatograms. A) Chromatogram of CS male flies. B) Chromatogram of *desat1* male flies. The peaks of interest are circled.

ii. Female Chromatograms

Eicosane was also used as a standard for the females. Figure 12 represents the chromatograms of CS and *desat1* female pheromones. The aphrodisiac pheromone, (Z,Z)- 7,11-heptacosadiene, was found in figure 12A to have a retention time of 22.18. This key pheromone was absent in the *desat1* female's chromatograms. When the mass spectra of each peak in CS and *desat1* females were analyzed and compared to each other, there were other difference between the compounds present. An example of this is the peaks that correspond to a retention time of 14.00. Both CS and *desat1* females have peaks 14.00 but the mass spectra differed. The mass spectra of the CS females, had a mass of 440 whereas the *desat1* females' had a mass of 267. Another place where they differed, is in the retention times themselves. After retention times of 14.50, there are no noticeable places of correspondence between the mutant and wild-type. When their mass spectra were analyzed, there were no similar masses found. These peaks were considered to correspond with these pheromones by comparing their mass spectra to the values determined by the National Institute of Standard and Technology (NIST).

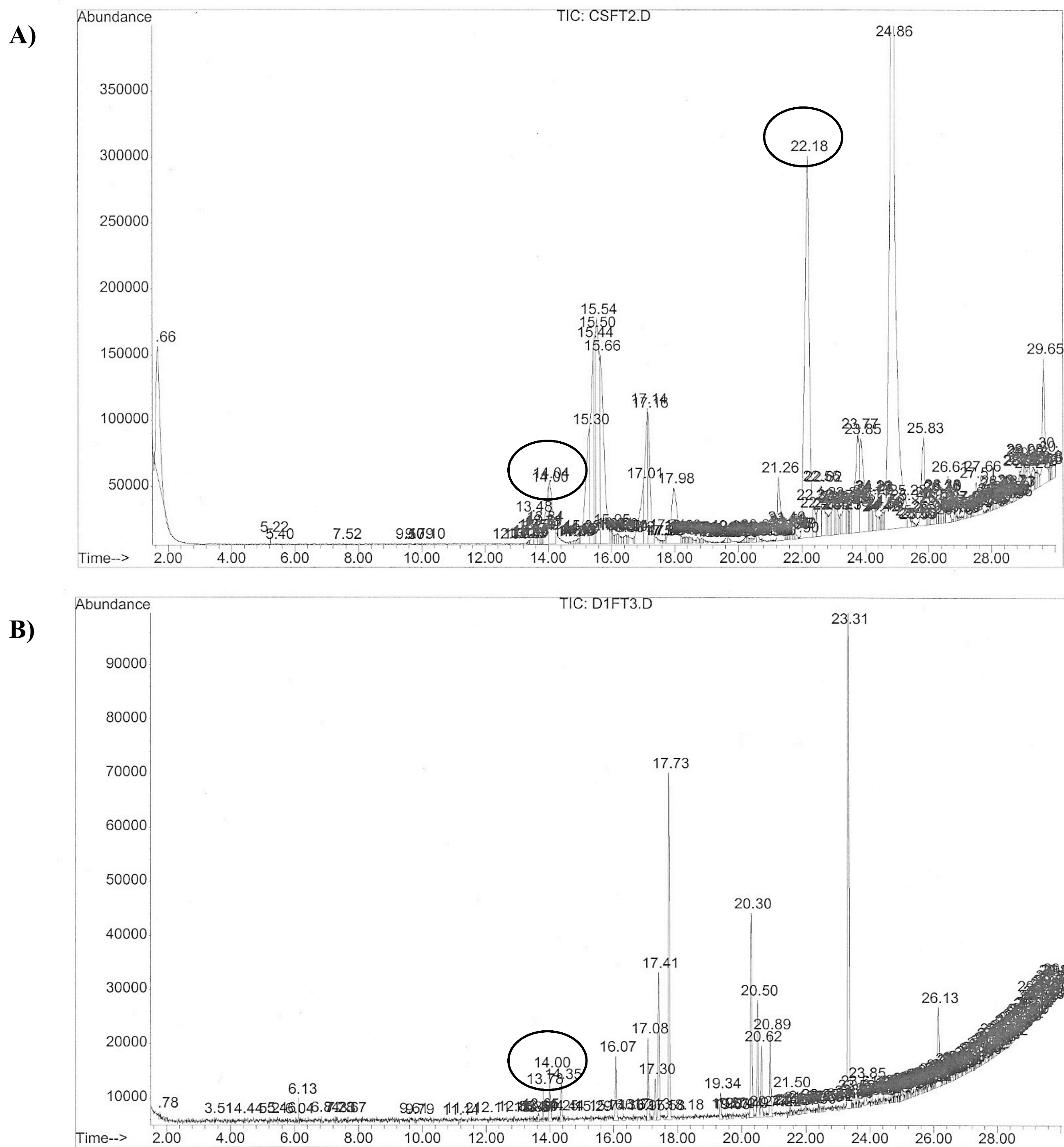


Figure 11. CS and *desat1* female chromatograms. A) Chromatogram of CS female flies. B) Chromatogram of *desat1* female flies. The peaks of interest are circled.

iii. Double Mutant

The double mutant was not successfully bred by the time of completion of this study. I was only able to breed up to the F3 generation. The crosses will continue so that someone can use these flies in the future.

DISCUSSION AND FURTHER DIRECTIONS

A. Courtship Assays

The goal of this study was to discover the root of why the *desat1* male will choose a *desat1* female over a wild-type, CS, female. *desat1* males are known to have a preference for male-male matings and will attempt to copulate faster than their wild-type counterparts (Houot et al 2012). To understand the underlying reason why they have this preference, researchers found that the primary cause is due to the mutants not producing the correct courtship stimulatory pheromones in detectable amounts (Ueyama et al 2005). *desat1* males are also known to almost always choose a *desat1* female over a CS female (Houot et al 2012). However, this is not readily understood. When researchers found that the *desat1* males prefer the *desat1* females, their behavior and the responses of other flies were analyzed. It was found that the females had the same courtship tendencies as wild-type females and that males had a normal response toward them (Houot et al 2012). Because they essentially behave and are treated by other flies like wild-type females, researchers did not conduct any further studies on the females.

From my observations of the courtship assays, I saw stereotypical courtship behaviors for each mutant. The CS males never displayed courtship behavior toward males of any kind and always showed courtship behavior towards females. This can be seen in figure 9 and 10 when looking at the courtship latency and courtship index. In figure 9A, the CL of males when presented with females was the highest with a value of 2.3 and the CL of males when presented with males was the lowest with a value of 1 which is the lowest score. This corresponds to what is expected of the wild-type. CS males also had a CI of 0 when presented with males and a CI of 1.45 when presented with females. These numbers correspond with what is expected of the wild-type males. When presented with females, they will attempt to mate and when presented with

males, they will ignore them. Also, when the CS males were presented with a *desat1* female, they responded to her as if she was wild-type.

My observations of the *desat1* males also matched what was recorded in Houot et al (2012). The CL values of *desat1* males when presented with females was 1.95. When they were presented with males, their CL was 1. The CL of *desat1* males when they're presented with both males and females had the highest score of 2. From my observations, and the observations made in the literature, this is the highest score because they males always initially attempted to mate with other males. After being rejected multiple times, the males would then mount the female in the chamber. In all my observations, the mountings were forced and the females always attempted to kick the male off their backs. This is most likely due to the female sensing that the courtship behavior was not directed towards her. One thing to note is that when CS males are presented with two females of any of my two genotypes, they will choose a female to court. If that female rejects him he will then restart courtship with the other female that did not reject him.

The CI values of the *desat1* mutants also correspond with what is found in the literature. *desat1* males had the highest CI value when presented with both males and females. The lowest CI value was the *desat1* male presented with a male. To better understand what this means, a better understand of what the values represent is needed. These values are largely due to the highest value in my scale represents copulation and not persistence or perceived "desire" of the male. If they were rated on persistence, number of continual attempts of mating, *desat1* males presented with males would have the highest value. They were not rated on persistence as there was no uniform method of measuring persistence found in the literature. Persistence is dependent on understanding the intricacies of female rejection. From observing the dance, in the early stages of courtship, for example orienting and dancing, the female tends to reject the male in

subtle ways. She might turn and face him or extend both wings to give herself space. In the literature, rejection is often seen as being running away, kicking the male, or increased movement of the female (Bontonou and Wicker-Thomas 2014, Ejima and Griffith 2007). As persistence depends on understand when rejection occurs and when the male reinitiates the courtship dance, persistence was not measured in this study.

The mutant males would always first attempt to mate with other males. Something that should be noted is that when two *desat1* males were in a courtship chamber, they were never receptive to mating at the same time. When they were observed, one of the males adopted the male role, or dominant role, in courtship and the other male adopted the role of an unreceptive female, or submissive role. The male in the dominant role will courtship and attempt to mount but the male in the submissive role would run away. When a *desat1* male attempts to mount a CS male, the CS will turn and face the mutant male and kick out at him. The submissive *desat1* male primarily runs away and will mainly kick the dominant male with his hind legs. Eventually, the dominant will realize that the potential mate is unresponsive. After a few minutes of ignoring each other, either one of the males will attempt courtship. They typically switch roles for approximately 30 minutes and then they ignore each other completely.

Observing courtship behavior had its own set of difficulties. The original camera that was used was not useful for recording high speed video. In order to have the camera record the rapid movements of courtship, the exposure had to be changed. However, when the camera was able to record courtship in real time, the image was very dark on the screen. To combat the darkness, intense light was shown on the flies. However, under intense light, the flies quickly became dehydrated. When they are dehydrated, they will begin to curl into a ball, defecate, and finally die. After seeing this occur repeatedly, I began to observe the matings using a dissecting

microscope and recording the matings using an iPhone 7 Plus. The phone camera was able to zoom in to capture their movements in real time. Natural light was bright enough for the iPhone to capture the matings.

B. Chromatogram

The CS and *desat1* male chromatograms both corresponded with what is found in the literature. The chromatograms of interest are the CS and *desat1* females' chromatograms. When looking at the peaks for the females, there were many discrepancies between the chromatograms. When comparing the molecular weights to the molecular weights found in the National Institute of Standard and Technology, the CS chromatogram corresponded with known pheromones. However, when comparing the *desat1* chromatogram to the CS chromatogram, none peaks after 14.50 had similar mass spectra. To reconfirm that the peaks all correspond to different compound, the values from the *desat1* mass spectra were put into the NIST database and none of the values matched.

There are many possible reasons that would lead to these results. The best-case scenario is that *desat1* mutant females do not express the gene *desat1*. Females have two double bonds in their aphrodisiac pheromones. They might not have both of the double bonds. From observing male behavior towards *desat1* females, it can be concluded that they are at least emitting a pheromone that specifies them as female. The worst-case scenario is that the peaks are different due to contamination. The same injection needle is used for everything done using the GC-mass spec. While protocols state that before and after each use, the needle must be clean, improper cleaning could have altered what was seen. Another possible place for error is the column itself.

The machine is typically only brought to maximum of 150°C. However, in my experiments, the column was brought to a maximum temperature of 300°C. This caused there to be a lot of noise due to chemicals that have never been burned off the column due to their size. It is possible that this noise has affected my trials. However, this is unlikely as compounds of similar sizes were not found in any of the trials involving either males or the CS female.

There were many difficulties encountered when using the GC-mass spec to analyze the pheromones. Initially the protocol in the Tompkins and McRoberts (1989) paper was solely used. However, there were several problems with the protocol. In the protocol, they suggested that one fly was sufficient to see peaks. They also said that injecting 1μL into the GC-mass spec was enough to see peaks. When I used their protocol, I did not see any peaks in their chromatograms. I performed the same protocol repeatedly and I sis not see a change in the results. I began researching protocols for analyzing pheromones from different animals and consulted multiple chemistry professors. Together, after many failed trials, we developed the protocol used in this study. Letting the samples sit for 12 hours was a unique condition that was not found in the literature. From performing multiple trials, 12 hours was the minimum amount of time where all the peaks could completely be seen.

C. Double Mutant

Due to time constraints, the double mutant was not successfully bred. This was largely due to mistakes made in the middle of the crosses that caused me to restart all crosses. The crosses will continue so that others in the lab can use these flies in the future for their experiments. The main reason why the double mutant wasn’t produced was due to the difficulty of the crosses. Figuring out the correct mating scheme took a large amount of time. Also,

perfectly coordinating when they would eclose and being sure of whether or not they are the mutants of interest or the progeny of the desired mutants. Due to this uncertainty, the crosses had to be restarted several times. This caused the double mutant production to be delayed.

D. Future Directions

If there was one element of my experiments that I would alter, it would be to have more time. If I had more time, there are many things that I would do. From observing the courtship assays, I saw that the females had very interesting but subtle responses to during mating. A more intense study of *Drosophila* female behavior would be a very valuable contribution to the field of understanding *Drosophila* courtship. Also, from observing the females, I learned that these subtle responses to courtship can determine the outcome of the mating. Also, from reading the literature, researchers repeated said the females are very uninteresting individuals to look at when studying *Drosophila* mating. They found these females so uninteresting that many modern *Drosophila* mating researchers do not even mention that females can be forced to mate. In researching the mechanics of their sexual behavior, I was only able to find one paper, Manning 1967, that listed the steps in a female being forced to mate. Looking for these responses can help us better understand mutant behavior. The *desat1* males were usually the ones who forced themselves on the females where the CS males were less likely to do so. Understanding females could illuminate us on the perception female *Drosophila* have of mutants.

Another element that I would change, is to do more chromatograms of the females. Due to time constraints, I was only able to analyze three chromatograms of each female genotype. While all three were consistent with each other, I am cautious to say that the pheromones are

indeed completely different in the *desat1* females. If I had more time, I would probably produce approximately 20 chromatograms. If after doing 20 trials, the chromatograms were consistent, I would definitively see that they are different. However, due to my small sample size, I can only be cautiously optimistic that the pheromones are different. Also, I wish I had more time to go through the NIST database and the literature in order to identify all the peaks found in the females' chromatograms. While I did find difference between them, I do not know if the difference is due to *desat1* females having saturated pheromones. This is most likely the cause for the differences but further trials must be performed in order to definitively state that.

The final element that I would change would be to have started breeding the double mutant sooner and to have had a more careful treatment of my earlier attempts at generating it. There is so little known about the behavior of that particular double mutant. Studying its behavior and pheromones would be incredibly novel research. My hope for the future is that someone will pick up my research and continue to study the complex behavior and pheromones involved in *Drosophila* mating.

E. References

References

Ågmo, A., and E. Ellingsen. "Relevance of Non-Human Animal Studies to the Understanding of Human Sexuality." *Scandinavian Journal of Psychology.* 2003. 44.3: 293-301.

Arbuthnott, D., T.Y. Fedina, S. D. Pletcher, D.E.L. Promislow. Mate choice in fruit flies is rational and adaptive. *Nature Communications*. 2017. 8(13953).

Blake, K. J., B. Rogina, A. Centurion, S.L. Helfand. Changes in gene expression during post-eclosional development in the olfactory system of *drosophila melanogaster. Elsevier*. 1995. 52:179-185.

Benton, R.. Sensitivity and specificity in *Drosophila* pheromone perception. *Cell*. 2007. 30:512-519.

Bontonou, G. and C. Wicker-Thomas. Sexual communication in the *Drosophila* Genus. *Insects*. 2014;5:439-458.

Ejima, A., and L.C. Griffith. Measurement of courtship behavior in *Drosophila melanogaster*. *CSH protocols* 2007: pdb.prot4847,pdb.prot4847.

Emmons, S.W. and J. Lipton. Genetic basis of male sexual behavior. *Wiley*. 2003. DOI 10.1002

Ewing, A. W. Functional-Aspects of Drosophila Courtship. *Biological reviews of the Cambridge Philosophical Society*. 1983. 58.2:275-292

Ferveur, J. Drosophila female courtship and mating behaviors: Sensory signals, genes, neural structures and evolution. *Current Opinion in Neurobiology*. 2010. 20:764-769.

Gailey, D. A., and J. C. Hall. Behavior and cytogenetics of fruitless in *Drosophila* melanogaster: different courtship defects caused by separate, closely linked lesions." *Genetics.* 1989. 121.4: 773-85.

Gailey, D. A., B. J. Taylor, and J. C. Hall. Elements of the fruitless locus regulate development of the muscle of lawrence, a male-specific structure in the abdomen of Drosophila-melanogaster adults. *Development*. 1991. 113.3: 879-90

Gaines, P., L. Tompkins, C. T. Woodard, J. R. Carlson. Quick-to-court, a Drosophila mutant with elevated levels of sexual behavior, is defective in a predicted coiled-coil protein. *Genetics*. 2000. 154:1627-1637.

Houot, B., S. Fraichard, R. J. Greenspan, J. Ferveur. Genes involved in sex

pheromone discrimination in *Drosophila melanogaster* and their background-dependent effect. *PLoS ONE*. 2012. 7:1-10.

Kallman, B. R., H. Kim, K. Scott. Excitation and inhibition onto central courtship neurons biases drosophila mate choice. *eLife*. 2015. 4(11188).

Kopp, A., I. Duncan, S. B. Carroll. Genetic control and evolution of sexually dimorphic characters in *Drosophila*. *Nature*. 2000. 408: 553-559.
Labeur, C., R. Dallerac, C. Wicker-Thomas. Involvement of *desa1* gene in the control of *Drosophila melanogaster* pheromone biosynthesis. *Genetica*. 2002. 114: 269-274.

Manning A. The control of sexual receptivity in female *Drosophila*. *Animal Behavior*. 1967.15:239-250.

Mostafa, T., G. El Khouly, A. Hassan. Pheromones in sex and reproduction: Do they have a role in humans?. *Journal of Advanced Research*. 2011.3:1-9.

O'Dell, K.M.C. and K. Kaiser. Sexual behaviour: secrets and flies. *Current Biology*. 1997. 7:6.

Pfaus, J. G. Frank A. Beach Award - homologies of animal and human sexual behaviors. *Hormones and behavior.* 1996. 30.3: 187-200.

Pfaus, J.G., T.E. Kippin, and G. Coria-Avila. What can animal models tell us about human sexual response?. *Annual Review of Sex Research*. 2003;14:1-63.

Ramanathan, S., J. Miewald, D. Montrose, M. S. Keshavan. Can age at sexual maturity act as a predictive biomarker for prodromal negative symptoms?. *Elsevier*. 2015. 164:35-39.
Rideout, E. J., A. J. Dornan, M. C. Neville, S. Eadie, S. F. Goodwin. Control of sexual differentiation and behavior by the *doublesex* gene in *Drosophila melanogaster*. *Nature Neuroscience*. 2010. 13:458-466.

Rybak, F., T. Aubin, B. Moulin, J. Jallon. Acoustic communication in *Drosophila melanogaster* courtship: are pulse- and sine-song frequencies important for courtship success. *Canadian Journal of Zoology*. 2002. 80: 987-996.

Siwicki, K. K., E. A. Kravitz. *Fruitless*, *doublesex* and the genetics of social behavior in *Drosophila melanogaster*. *Current Opinion in Neurobiology*. 2009. 19:200-206.

Symonds, M. R. E., B. Wertheim. The mode of evolution off aggregation pheromones in *Drosophila* species. *European Society for Evolutionary Biology*. 2005. 18: 1253-1263.

Thistle, R., P. Cameron, A. Ghorayshi, L. Dennison, K. Scott. Contact chemoreceptors mediate male-male repulsion and male-female attraction during *Drosophila* courtship. *Cell*. 2012. 148:1140-1151

Tompkins, L., S. P. McRobert. Regulation of behavioral and pheromonal aspects of sex determination in *Drosophila melanogaster* by the *sex-lethal* gene. *Genetics Society of America*. 1989. 123: 535-541.

Ueyama, M., T. Chertemps, C. Labeur, C. Wicker-Thomas. Mutations in the Desat1 gene reduces the production of courtship stimulatory pheromones through a marked effect on fatty acids in *Drosophila melanogaster*. *Elsevier*. 2005. 35:911-920.

Vakirtzis, A. and S. C. Roberts. Mate quality bias: sex differences in humans. *BioOne*. 2010. 47:149-157.

Weigmann, K., R. Klapper, T. Strasser, C. Rickert, G. Technau, H. Jackle, W. Janning, C. Klambt. FlyMove- a new way to look at development of *Drosophila*. *Trends in Genetics*. 2003. 19: 310-311.

Yamamoto, D. Genetic dissection of sexual behavior in *Drosophila melanogaster*. *Annual Reviews*. 1997. 42:551-585.