Role of Sensory Input in the Courtship Strategy of Male *Drosophila Melanogaster*

Over the last half century, the courtship behavior of male *Drosophila melanogaster* has served as a model for the study of a diverse range of biology processes. In more recent years, a stronger understanding of the sensory cues underlying initiation of courtship has emerged. Much less is known, however, regarding the function of the two components of the male's courtship song and their dependence on female behavior. Here, I show that the courting male dynamically adjusts the relative proportions the two songs as a function of female locomotion; this phenomenon is termed “courtship strategy.” Males deficient for olfactory processing as well as males with poor visual acuity have non-wild-type courtship strategies. The specific manifestations of these differences are dependent upon the deprived sensory modalities. Results suggest a mechanism of song production in which the wing is invigorated by two CNS signals. One yields sine and pulse stochastically in nearly equal proportions and the other yields only pulse. The activity of these two signals are modeled as functions of sensory input in a way that recreates the courtship strategy observed for both wild-type and mutant flies. The proposed circuit by which courtship strategy arises offers potential insight into the mechanisms by which behavior is dynamically adjusted in response to changing sensory cues. Furthermore, courtship strategy offers a tractable model for the study of higher-order sensory integration in a relatively simple organism.
1. Introduction

1.1 Introduction to *Drosophila* Courtship

In the wild, mate selection is critical to ensuring the survival of a species. The processes by which a mate is selected must obey the utmost efficiency and effectiveness. Given such a requirement, animal courtship has enthralled evolutionary biologists for many years. Among the most studied courtship models is that of the fruit fly, *Drosophila*, especially within the species *melanogaster*. In the laboratory, *Drosophila* courtship is quickly observed and easily quantified under experimental conditions. As such, decades of research in this field have uncovered not only the evolutionary significance of this behavior but also a burgeoning understanding of the neural processes and computations that govern it. Continued research promises to yield many insights into how sensory input becomes translated into motor output, a topic whose significance to the neuroscience community is not easily exaggerated.

When placed in a chamber with a conspecific female, the *Drosophila* male does not generally hesitate to initiate courtship. The steps of courtship follow a necessary sequence and are readily observed by the trained eye (reviewed by Hall 1994): First, the male recognizes the female and orients towards her then circles her if she is immobile or begins to follows her otherwise; the next step involves tapping the female’s abdomen with what functionally serve as the male’s taste receptors; after this, the male extends a wing and vibrates it to produce song—this step conveys the most information and is arguably most essential to efficient courtship; after a variable amount of singing, the male licks the female’s genitals and immediately follows by attempting copulation; if he is unsuccessful, he repeats the cycle, starting anew with song production. The female’s role appears to be more passive but is certainly important; she is unreceptive by default and,
over the course of successful courtship, becomes receptive to copulation. The cues by which she signals receptivity are not fully understood. Identification of such signals and their relative salience to the male are a matter of current investigation.

The aforementioned song—referred to as the “love song”—is typically understood to represent the most important transaction of information between the male and the female. The love song is produced by male unilateral wing vibration and can be broken down into two subtypes (the following information relates to *D. melanogaster* and is not true for all *Drosophila* species): Pulse song and Sine song. Pulse song is characterized by trains of temporally punctuated “pulses” which typically consist of only a few cycles; one of which typically has a noticeably larger amplitude than the others. The amount of time in between these distinct spikes is termed the interpulse interval (IPI) and is distinct from the frequency of the signal (*ALT et al. 1998*). Sine song is much simpler. It is not characterized by dynamic pulsation or patterned amplitudes. Rather, it is most easily characterized as a low hum; thus, the defining characteristic of any given sine train is its carrier frequency (*WHEELER et al. 1988*). The respective roles of these two song types will be discussed later.

1.2 Mechanisms of Song Production
With the goal of understanding the neural substrates of song behavior, it is essential to give consideration to the features of song production over which the central nervous system has control. Though relatively few studies have attempted to answer this question, those that have done so provide strong evidence for the notion that song parameters (i.e. IPI, intrapulse frequency, and sine carrier frequency) are the product of pattern-generating musculature rather than central control. An early investigation into the
neuromuscular basis of courtship song (Ewing 1979) demonstrated a distinct set of wing muscles whose firing patterns were shown to underlie wing movements known to produce song. Only a small difference in the set of muscles used for sine and pulse song, respectively, was observed. The wiring of inputs to these muscles supports the idea of relatively simple signals from the brain. The more complex orchestration of muscle contractions is likely achieved by local proprioception (Trimarchi and Schneiderman 1994). A recent investigation of the anatomical substrates of song production has specifically demonstrated that the acoustic components of the courtship song are not a consequence of coordinated input from the CNS (Clyne and Miesenbock 2008). In this study, direct stimulation of a sexually dimorphic bundle of axons descending onto the muscles controlling wing vibration was able to yield coherent courtship song of both types. Thus, a pattern-generating circuit likely executes singing, while central brain regions simply activate or inhibit said execution.

Considering the evidence supporting song characteristics as a function of musculature, the primary role of the CNS in courtship song seems to be initiation and suppression of courtship activity. Two separate and methodologically distinct studies support the idea that the putative on-off neural command region involves a site integrating multiple sensory modalities (Broughton et al. 2004; Hall 1979). The more recent of the two specifies the lateral posterior protocerebrum as said site, though it is likely not the only courtship-relevant site of sensory integration. As discussed by Clyne and Miesenbock (2008), available data regarding central and motor control over production of pulse song versus sine song likely adheres to one of two models: (1) the brain signals either sine or pulse song directly, each through its own separate input or (2)
the brain has an "indiscriminant" signal to sing with no song type specified and a separate "pure" signal to control a specific parameter of the motor pattern which biases the musculature towards a distinct song type. Though the latter model is favored, neither have direct experimental validation. In either case, the inherent relevance of these findings to sensory integration and courtship behavior is the simplicity of the output from the male brain. While representative of an obviously complex network, the pulse and sine components of courtship song also represent the direct outcome of a neural decision of an ultimately binary nature.

1.3 Respective Roles of Pulse Song and Sine Song

Understanding the significance of song production decisions (that is, pulse song versus sine song) can only be done by placing production in the context of the courting male's goal—to copulate. Actions to achieve these goals are thus ultimately a product of the female's criteria for mate selection. As such, song production is best understood through such a lens. Discerning the elements of female criteria has typically been the goal of playback experiments, in which the behavioral response of the female to the courting male is examined under experimenter-controlled exposure to simulated song. Since a male with amputated wings will still court, this gives the experimenter the freedom to manipulate elements of the love song in order to tease apart the relative function of its constitutive components. In playback experiments, the receptivity-increasing effects of courtship song on females have been shown to be most prominent for conspecific songs, suggesting a role of species identification (RITCHIE et al. 1999; TOMARU et al. 2000; TOMARU and OGUMA 2000). In support of that notion, IPI is found to be quite stereotyped for different species of Drosophila (MOULIN et al. 2001; WHEELER et al.
1988) and impressively invariant for different geographically isolated populations of *D. Melanogaster* (RITCHIE et al. 1994). The carrier frequencies of both pulse and sine song do not substantially affect female receptivity. Furthermore, either song alone (when played at the typical frequencies) enhances receptivity, though the effect is stronger for pulse song (RYBAK et al. 2002a).

As a pre-courtship stimulus (played to females immediately before males begin courtship) sine song, but not pulse song, decreased copulation latency relative to white noise. Played mid-courtship, pulse song significantly decreased copulation latency. The effects of this were significantly more robust when the IPI was conspecific with the *D. Melanogaster* female. With decreased copulation latency as a measurement of increased receptivity, the interpretation arises that pulse song identifies the species of the male and sine song generally enhances receptivity (VON SCHILCHER 1976), which seems relatively consistent with the above studies. In general, there is little doubt regarding the notion that pulse song serves to identify the species of the male. The information conveyed by sine song, however, is less resolved. Evidence exists both for (VON SCHILCHER 1976) and against (ROBERTSON 1982) the idea that sine song increases female receptivity in a manner proportional to its aggregate duration during courtship.

Chemical signals from the male also influence courtship success (RYBAK et al. 2002b), offering some explanation for why a small number of control males in playback experiments (males with no wings and no experimenter-added song) are still successful in copulation. While they both serve to enhance receptivity, neither chemical signals nor acoustic signals alone are 100% indispensable for courtship success; although, in the absence of both of these signals from the male females will not copulate.
Overall, playback experiments suggest, rather anticlimactically, a simple
redundancy of function between pulse song and sine song (and male pheromones).
However, these investigations have failed to examine male song behavior in the context
of female behavior and thus ignore the very likely possibility that the male uses female
cues to alter the pulse/sine profile of his love song. Such a profile is what I refer to as
“courtship strategy.”

A potential and intuitively salient cue regarding receptivity that the female could
provide to the male is movement. The transition from courtship to copulation requires
that the female be still. The progressive decrease in female movement over the course of
successful male courtship has been thought to reflect the simultaneous increase in the
female’s receptivity (von Schilcher 1976). Simulated courtship song was in fact shown
to reduce female locomotion in the presence of a wingless male, supporting the notion
that females’ movement signals her receptivity (Kowalski et al. 2004). Considering the
requirement of stillness when initiating copulation and the well-understood role of
courtship song in increasing the receptivity of the female, it is perhaps not surprising that
that which promotes copulation reduces movement. What is potentially interesting, and
still unexplored, is the extent to which the male integrates information of female
movement into his own behavior.

It seems feasible to believe that the limited understanding of the respective roles
of pulse and sine song is a consequence of playback experiments’ failure to integrate the
simulated song into the context of female behavior. That is to say, the “roles” of pulse
and sine song are not necessarily static but perhaps change over the course of courtship.
Along such lines, a main purpose of this thesis project is to explore how, if at all, female
movement yields a dynamic adjustment of the male's courtship strategy. In single-pair recording situations, it is possible to directly quantify both female movement and male song production. Understanding this relationship will not only help to elucidate the respective roles of sine song and pulse song but will also potentially uncover a model through which the integration of sensory input and behavior can be further explored.

1.4 The Importance of Sensory Cues and Courtship

It should go without saying that males do not court constitutively. Just as females require a specific set of stimuli from the male to accept copulation, the males require a specific set of stimuli from the female to initiate courtship. To date, most, if not all, studies of sensory modulation of male courtship behavior have focused on courtship initiation or vigor. While the proposed experiments are intended to focus on behavior that specifically comes after "initiation", there is likely overlap between sensory input governing initiation and sensory input governing song strategy. Thus, findings regarding sensory integration and general courtship bear strong, albeit indirect, relevance to the topic of sensory integration and courtship strategy.

The influence of sensory processing on male courtship behavior is multimodal in nature, involving visual, gustatory, olfactory, and, to a small extent, auditory input. As with all the forms of sensory information used by males, visual input is dispensable for courtship initiation if other modes of sensory processing are left intact. Nevertheless, visual information certainly plays a role in stimulating courtship behavior and effective courting. In the most apparent sense, visual processing is required for effective orientation towards and tracking of the females. However, the involvement of visual input in courtship conditioning suggests that it significantly interacts with other sensory
modalities in the control of neural commands regarding courtship (JOINER and GRIFFITH 1997).

Gustatory processing involves forms of chemosensory signals received by direct contact between the male and the female. This is generally thought to involve non-volatile cuticular compounds (reviewed by FERVEUR 2005). Genetic modification of long-chain pheromone profiles have been shown to affect male courtship intensity, with female-specific 7,11-diene pheromones stimulating this behavior (FERVEUR and SUEAU 1996). One study specifically ablating neurons expressing a putative gustatory pheromone receptor observed direct consequences on courtship behavior (BRAY and AMREFIN 2003). Specifically, courtship initiation and vigor were diminished, implicating a stimulatory role of the female pheromones specifically accessed by the male during the tapping stage of courtship.

Pheromones do not only transmit information through gustatory receptors but also do so via olfaction. In general, pheromones that act as substrates of olfactory receptors have a higher volatility than those putatively identified as gustatory substrates. Ostensibly, female pheromones of this type are more easily deposited or shed from the body (FERVEUR 2005). The role of the fruitless (fru) gene in establishing sexually distinct courtship behavior has been researched heavily. There is a large body of work demonstrating fru as a transcription factor with courtship-circuit-specific expression in the fruit fly CNS (reviewed by MANOLI et al. 2006). An experiment in which fru-expressing olfactory neurons were specifically targeted and ablated provides compelling evidence for a role of olfaction in courtship behavior (STOCKINGER et al. 2005). Stockinger and colleagues managed to demonstrate a reduction in courtship intensity as a
consequence of ablating courtship-specific olfactory receptor neurons (ORNs). Furthermore, they found that females expressing the male *fru* protein—which causes females to engage in male courtship behavior—would court males expressing a female pheromone profile and that this behavior was heavily reduced by silencing *fru*-expressing ORNs. Recently, cis-vaccenyl acetate (cVA) was identified as an aversive pheromone. It is synthesized by males specifically and taken up by mated females, providing them with a distinctive pheromone profile that has been shown to mediate male courtship conditioning (EJMA et al. 2007). Interestingly, cVA does seem to have an aversive effect on courtship beyond that seen in learning experiments, but such an aversion to cVA remains highly context-dependent (GRIFFITH and EJMA 2009a).

An understanding of the functional circuitry linking sensory perception and courtship behavior is a very feasible goal for continued investigation within this field of research. Teasing apart the relative contributions of gustatory and olfactory input, however, has been challenging given the relative similarity between chemosensory substrates. With one of the main putative differences between gustatory and olfactory perception being the volatilities—or lack thereof—of respective substrates, experimental distinction between taste- and smell-based cues is understandably difficult. Nevertheless, the aforementioned experiments have helped to identify the importance of each form of chemosensation in courtship behavior. A more resolved understanding, however, is still desirable. When considering the sequential steps in which the male performs courtship, it is important to note the degree to which something such as substrate volatility can influence the array of perceptual systems activated at any given step of courtship. In the context of his dutiful following, the courting male’s proximity to his female target is
logically linked to her locomotion. In the context of cue-specific-volatility, this dynamic locomotion could create an equally dynamic set of cues by which the male is able to assess female receptivity.

With such considerations in mind, the other main goal of this project is to evaluate how sensory information dictates courtship strategy. In many ways, this is an extension of how locomotion affects strategy. That is, female movement is likely to be a very salient behavioral cue for the courting male: it provides sensory information accessible to visual, gustatory, olfactory, and auditory systems. Furthermore, which sensory systems are engaged and how they are engaged is likely to be a direct consequence of the degree of female locomotion. When the female moves at a rapid pace, the distance between the male and female logically increases, potentially making the most salient modality vision. On the other hand, when the female is more still gustatory perception may become more salient. Lastly, female locomotion is thought to correlate—at least roughly—with her receptivity. It seems beyond coincidence that the sensory modalities understood to be engaged during courtship so neatly correspond to those dynamically activated by this indicator of receptivity. With an arsenal of genetic approaches by which the researcher can manipulate the sensory capabilities of the fruit fly, it will be highly possible to test the relative contributions of these sensory modalities to the courtship strategy of male D. melanogaster. I seek to answer how, in a general sense, the modalities of vision, gustation, and olfaction give rise to male courtship strategy. This will require quantifying male strategy (i.e. the relative proportions of the two song types in a given window of time) in the context of female behavior (i.e. amount of female movement in the same window). Furthermore, it will require an examination of
how the absence of a given sensory input interferes with normal strategy. Positive results from this investigation will potentially yield a direct connection between sensory input and behavioral output and, thus, a relatively comprehensive model for the biology of sensory-motor transformation and decision-making.

Over the course of its research, *Drosophila* courtship has yielded many insights into diverse biological processes. What started as an interesting model of evolutionary diversification has evolved into a fairly ideal model for understanding the biology of behavior (Dickson 2008). *Drosophila* courtship is a relatively complex behavior of a relatively simple organism, making it a promising target for studies seeking to understand how sensory information is integrated for the purposes of decision-making. Following clear demonstration of the multimodal nature of involved sensory systems (reviewed by Griffith and Ejima 2009b) and the context-dependence of these stimuli on male courtship (Ejima et al. 2007; Joiner and Griffith 2000), the task at hand now becomes achieving an understanding of what sensory cues influence specific sub-behaviors and, ultimately, how a complex neural network achieves the transformation from sensation to action.
2. Materials and Methods

2.1 Genetic Manipulations

For all behavioral experiments, the wild-type control flies were Canton-S (CS). As the control flies, they had all sensory modalities intact. To investigate the effects of disrupted processing on courtship strategy, I also examined the courtship behavior of flies with a deleted Or83b gene (Or83b\(^2\)) and their genetic “controls” (Or83+). The Or83b gene mediates localization of chemoreceptor ion channels to the membrane of ORNs. Because of the gene’s broad expression throughout ORNs, its deletion has the capability to deprive the flies of olfactory input (LARSSON et al. 2004). In the Or83b\(^2\) genotype, the Or83b gene is deleted by inserting a mini-white sequence into it. This line is genetically w, meaning that all eye color comes from mini-white insert. As described in Larsson et al. (2004), Or83+ “control” flies, which are also genetically w, have the mini-w construct randomly inserted somewhere on the third chromosome. While this spares the Or83b gene, it should impinge upon visual acuity such that Or83+ flies have diminished vision similar to the Or83b\(^2\) line. Manifestations of this eye phenotype on behavior will be discussed later. All experiments discussed hereafter focus on the behavior of male flies within these three strains.

2.2 Recording Setup

Courtship behavior was studied under single pair conditions. All experiments consisted of song recording and visual recording to allow comparison between song production and courtship events. All recording conditions used an Aktogen Courtship Song Chamber, a 3mm tall cylindrical chamber with a diameter of 12 mm on bottom and 14 mm on top, equipped with a movable barrier to grant control over when the flies are exposed to one another. The chamber rests on top of a microphone platform housing two
specialized Aktogen microphones, which feed into an amplifier and from it into a computer. Also connected to the computer is a video recording device situated just above the transparent chamber. Video and audio were recorded simultaneously with QuickTime Pro. All recordings took place in a sound-insulated room with gain settings held constant at a level determined to be ideal.

Before each pair was tested, the recording chamber was dismantled, cleaned with 70% ethanol, dried, and reconstructed. Occasionally, a portable heating device was used in between trials to keep the temperature around the chamber near 25°C Celsius. The test fly and his courtship target were loaded separately into divided regions of the chamber. After removing the barrier between the flies, I listened to and watched the pair until love song was heard or overt courtship behavior was observed. Recording began immediately after such an observation was made. The male was allowed to court the target for a fixed duration of time (typically, 15 minutes), after which recording was terminated. In the event that copulation occurred, recording was terminated accordingly. Within a given experimental paradigm, the goal was to record approximately 1 hour of total courtship per strain; because of differing baseline intensities and rates of copulation success, this required a different number of flies per strain, though generally as little as 5 to 10 flies were sufficient.

After recording, audio extracts of the courtship files were pre-processed using Amadeus Pro. This involved first employing a band-pass filter to remove all frequencies between 0 and 120 Hz. Such a step was required to eliminate the constant noise from the nearby computer and most of the periodic noise from outside the room. Following the band-pass filter, a background noise elimination tool was used to filter out all sound
matching a user-selected sample of pure background noise. While relatively little processing was needed to improve the signal associated with pulse song, sine song was often difficult to hear behind the noise. These manipulations greatly enhanced the signal quality of sine song, making it clearly audible and distinguishable.

2.3 Experimental Designs

The project consisted of two major experimental paradigms: (1) test males courting virgin CS females, and (2) test males courting virgin CS males. For all experimental designs, all flies were collected 0-6 hours after eclosion and raised in isolation—each fly was raised in its own test tube containing food medium with yeast—and kept on a 12h light/dark cycle. On the day of recording all flies were 5 days old. Recording sessions began near the early stages of the flies’ entrained light cycle since behavior was examined under normal room lighting.

2.4 Data Analysis and Modeling

Pre-processed songs were scored by hand using Amadeus Pro. In general, pulse and sine are clearly distinguishable when there is little or no background noise; in such cases, either listening to the sound file or looking at the sound trace was sufficient to identify song. On occasion, however, the identification was less absolute. Since bouts of pulse song were often surrounded by brief periods of non-song noise and since sine song is most abundant before and after pulse bouts, sound surrounding pulse bouts that partially resembled sine song were difficult to classify. This occasional ambiguity likely served as the source of almost all false negatives and false positives. In instances of said ambiguity, the decision of whether to score the sound in question was based on information provided by analyses of spectral composition. Admittedly, the decision
criteria surrounding said analyses were not strongly defined and required a subjective judgment. In general, however, the criteria included the presence of a prominent and at least somewhat narrow (no more than 20-30 Hz wide) spike in the intensity of the sound at a frequency between 125 and 200 Hz.

When measuring courtship strategy, in each full 30-second window of courtship in which at least 2.5 seconds of song were measured, percent pulse song (%P) was calculated as the fraction of time spent singing pulse song during a given window over the total time spent singing. When measuring production of specific song components, time spent singing each song was reported for each full 30-second window regardless of the total amount of song produced. Curves are often presented as sliding window averages; obtaining the sliding window average involves grouping the data spanning 3 adjacent movement levels into a single window and generating an X and Y coordinate from the average movement level and average production variable, respectively. The window “slides” over the data and generates n-2 points, where n is the number of movement levels containing data for a given set. Standard error bars associated with these curves use all the data in the window.

Female movement was quantified using the video captured during the courtship recording. A transparency with a vertical line drawn across it was placed over the computer screen and the video file of a given pair was opened and positioned such that the vertical line divided the chamber in half. For each full 30-second window, movement was quantified as the number of times the female crossed the vertical line. It was not enough for the female to cross the line and immediately turn around. Rather, two successive crossings were each counted only if the female reached a designated “reset”
area on either side of the line in between said crossings. Females tended to move in circles around the perimeter of the chamber and, while their movement was not specifically restricted to this pattern, this tendency certainly increased the extent to which the number of crossings is representative of female locomotion in a given time window.

As discussed in Section 1, a key variable that is likely associated with female movement is the distance between the female and the courting male. For a more complete analysis of the relationship between courtship strategy and distance, I created an object-tracking program using MATLAB 2008b. The program compares each frame of video to the average of the full video file and labels discrete pixel clusters whose absolute difference is sufficiently greater than the background. The program is able to recognize flies based on their sizes and the inherent assumption that objects move in continuous paths. At each frame, distance was measured as the Euclidean distance between the flies’ centers of mass. Distance is reported in Arbitrary Units, as it was normalized to the distance in pixels separating two distinct structures in the chamber, the length of which changed as a result of slight differences in camera positioning during recording.

MATLAB was also used to automate the song annotation process in order to examine the relationship between song production and distance with higher temporal resolution. The program was designed to objectively identify segments of the song file as pulse, sine, or (by default) silence. I designed the criteria based on my own and compared the results of the program’s analysis to the results obtained when scoring data by hand. Once the criteria were established such that the program returned results very close to my own, it was used to find the exact time points at which the male produced either pulse or sine song. With this information available, it was possible to examine the temporal
correlation between fly distance and bout onset for each type of song, thereby providing an indication of the ideal distance stimulus for each type of song within each fly strain. This method of analysis is analogous to a Spike-Triggered Average, but could be thought of as a Bout-Triggered Average. For every separate bout of a given type of song for a given fly strain, the distances separating the fly from 5 seconds before to 3 seconds after the onset of the bout are stored. For each type of song for each type of fly, the average of these distance curves serves as the representation of the ideal distance stimulus.

Following very careful investigation of the data, the proposed system underlying the sensory-motor transformation involved in male courtship strategy was modeled using MATLAB. The model creates a semi-random pattern of movement intensity as a function of time and a corresponding pattern of distance, which was modeled as a random process influenced by movement. At each time point, activity patterns in various modalities were modeled as a function of distance and/or movement, and their states at the previous time step. Once theoretical data recapitulated data observed with CS males, the saliency coefficients and/or activity patterns of given modalities as functions of movement or distance were modified. These modifications were not arbitrary; rather, they were entirely analogous to sensory deficits conferred (or thought to be conferred) by a given fly's genotype. The details of the sensory integration and these modifications will be discussed later.
3. Results

3.1 Homosexual Courtship

Going into this project, there existed potential complications brought about by the ectopic expression of mini-w in the genetically modified males. There is a well-documented tendency for ectopic expression of mini-w to result in homosexual courtship among *D. melanogaster* males in white light (Anaka et al. 2008; Svetec et al. 2005; Zhang and Odenwald 1995). This type of courtship is suppressed in the dark, suggesting that it is due to an abnormally processed visual cue (Hing and Carlson, 1996). Animals that are mutant at the w locus and contain a mini-w transgene have lighter eye color than wild type flies. This decrease in pigment results in less shielding of individual ommatidia from off-axis light and a decrease in visual acuity. Given that the white protein is also involved in trafficking of neurotransmitter precursor molecules, the fact that it's ectopic expression can alter the courtship behavior of male fruit flies is of obvious concern. That is, the possibility exists that this marker may fundamentally alter the pheromone and/or visual cues the male prefers. Furthermore, the mini-w construct does not necessarily affect the Or83+ males and Or83b males equally, if at all, since its insertion into the chromosome occurred at different locations. It is clear from many observations of transgenic lines that contain mini-w (L. Griffith, personal communication) that homosexual courtship is not an inevitable consequence of having this type of transgene, but rather is associated with specific insertions sites. Thus any difference between Or83b^2^ and Or83b^+^ would not be surprising. While it was known that the sensory capabilities of the CS males are normal (that is, wild-type) and the olfactory capabilities of the Or83b^2^ males are absent, the visual capabilities of the Or83b^2^ and Or83+ flies were not obvious.
For such a reason, assessing the tendencies of the different males to court other males seemed a reasonable avenue for exploring the nature of their visual deficits. Rather than characterizing the "courtship strategy" of the males, the courtship index (CI) was used as a measurement of courtship behavior. In this case, the CI is the fraction of time in the 10 minutes after exposure to one another spent courting. The male is considered to be courting when any of the stereotyped courtship behaviors are observed, including chasing.

As one would expect, the CI of all wild-type males was 0 (data not shown). For experimental considerations, all pairs consisted of one test male and one courtship object, though when testing wild-type homosexual courtship, both test and object were identical, making the distinction as test and object arbitrary and ultimately meaningless. As such, the likelihood of observing wild-type homosexual courtship under this set up should be at least doubled. With such a consideration in mind, the complete absence of homosexual courtship among pairs of virgin CS males bolsters support for the notion that the profile of sensory information that one wild-type male receives from another is not normally conducive to courtship.

Homosexual courtship was observed among Or83 flies.

Figure 1. Homosexual Courtship Behavior of Mutant Flies. Courtship indices are shown for each group of mutant flies. Error bars represent SEM. The difference between the fly strains is significant (p < .05).
The mean CIs for these two fly strains are depicted in Figure 1. Though courtship towards other males was not particularly abundant among either strain, the Or83+ males exhibit homosexual courtship significantly more than the Or83b² males (p < .05). These results almost certainly indicate a differential effect of the mini-w construct on these two strains. Were the effects the same, the combined deficits in vision and olfaction would likely result in a higher homosexual CI among the Or83b² males, considering the known inhibitory role of male pheromones. A study by Liang Yin Hing and Carlson (1996) reported that homosexual courtship among males misexpressing mini-w is a light-dependent phenomenon such that in the absence of light mutant and wild-type males had nearly equal (and very low) homosexual CIs. This invites the speculation that altered courtship behavior among males with ectopically expressed mini-w is a consequence of impaired sensory capabilities rather than a partial re-wiring of the courtship sensory-motor circuit. The preferred interpretation of this data is that Or83+ males are specifically deficient in aspects of visual processing, considering that behavioral differences relative to wild-type flies are only accountable for by mutations best understood to affect vision. Furthermore, the presence of the mini-w construct in the Or83b² genome should not be considered to confer similar deficits to this strain. Thus, for the purposes of this project, Or83b² males will be considered to be incapable of receiving olfactory information but functionally normal in terms of vision.

3.2 Courtship Strategy and Female Movement
With the sensory capabilities of the male flies more clearly defined, it became possible to examine the effects of sensory deficiencies on courtship behavior. The main experimental paradigm of this project examined the courtship behavior of test males in
Figure 2. Courtship Strategy of Male Flies. Raw courtship data is plotted according to the number of crossings within a given window and the percentage pulse song within the same window for wild-type (A), Or83+ (B), and Or83b<sup>C</sup> (C) males. Dotted lines were obtained from a linear regression analysis.

the presence of virgin CS females. The key variable was courtship strategy, which is quantified in terms of percent pulse (see Section 2.4). In wild-type flies, there is a very strong correlation between female movement and %P (Fig. 2A); the correlation coefficient from the linear regression analysis is $r = .6243$ ($p < 1\times10^{-5}$). There is also a
strong correlation for Or83+ flies (Fig. 2B) though the relationship is not as steep; the correlation coefficient from the linear regression analysis is $r = .4055$ ($p < 1 \times 10^{-4}$). As for the Or83b² flies, however, there is no correlation (Fig 2C). One can clearly see that little, if any, adjustment of courtship strategy occurs in response to a change in the female's movement. The correlation coefficient ($r = .1606$) is non-significant ($p > .05$).

A clearer view of how the male changes his courtship strategy in response to female movement can be achieved by consolidating the data using a sliding window average (Fig 3). Looking at the data in this format, several noteworthy observations arise. First, the relationship between %P and movement is highly linear in wild-type male flies. This data gives considerable legitimacy to the arguments that (1) the roles of sine and pulse are best understood in the context of female behavior and (2) that the male dynamically changes his behavior in response to some sensory cue or set of sensory cues that changes as a function of female movement. Second, olfaction seems to play a very considerable role in the male’s ability to update his song production in accord with the female’s behavior. Only at very high levels of
movement do the Or83b² males seem to shift their song production towards pulse song and away from what appears a stochastically equal mix of both. Third, while the %P curves of the Or83+ and CS males agree at higher levels of female movement, they seem to be rather different when the female is moving slowly. Specifically, the Or83+ males are more biased towards pulse song at these movement levels than wild-type males.

3.3 Song Production and Female Movement

These different strains of flies vary not only in aspects of their courtship strategy but also in their general song production. Figure 4 depicts the sliding window average of total song production as a function of female movement. Considering the very likely possibility that the Or83+ flies possess visual deficits, it is perhaps not surprising that they tend to produce less song. That is, they are feasibly less receptive to some female cue, which are generally thought to be excitatory. Of particular interest, however, is the very dramatic increase in raw song production of the Or83b² flies. Research measuring

---

**Figure 4. Total Song Production as a Function of Female Movement.** Sliding window averages for total song production at varying levels of female movement. Wild-type data is shown in blue, Or83+ in red, and Or83b² in yellow. Error bars represent SEM.
the courtship intensity of male *Drosophila* has shown that olfaction promotes courtship. These results do not contradict those of previous studies, as courtship intensity is not being measured. However, this is clear evidence that olfaction likely serves a role in the inhibition of song production.

Examining the production curves of sine and pulse song specifically within each strain provides an even more comprehensive picture of how courtship strategy might arise. As one can clearly see, the progressive increase in %P among wild-type males as a function of increasing female movement seems to occur as both an increase in overall pulse production and a waning production of sine song (Fig 5A). A comparison between the wild-type data and that of the mutant flies is not straightforward. The mutant and wild-type flies share little in common with regard to production curves (Fig 5). Indeed, little similarity, if any, is preserved among this data.

These data are easiest to interpret when considering two potential models of song production, which were described in Section 1. In one model, there is distinct central control over both sine and pulse song such that each is produced from a separate central command. In the other, central input is able to (1) initiate indiscriminate signing such that pulse and sine are produced only probabilistically and (2) influence some particular state of wing musculature to bias production towards one type of song. While the first model may seem to be more immediately intuitive, the data presented thus far do not easily fit into its predictions. Rather, the data is largely consistent with the predictions of the second model, as I will soon describe, and take a more meaningful shape when considered through its lens.
Consider first the courtship strategy data (Figs 2 and 3). While there are individual 30-second windows in which sine production exceeds that of pulse (such that $\%P$ is less than 50) there is little evidence to suggest that, for any of the fly strains, there are female movement levels that cause the male to weight his production towards sine
song. However, there are undoubtedly female movement levels that cause the male to weight his production towards pulse song. For wild-type flies, the strategy observed at the two extremes of female movement (allowing some interpolation of the trendline beyond the actually observed data) is very consistent with the two central commands described in the second alternative of the model. That is, at very low levels of female movement, the indiscriminant signal seems to be most—if not entirely—active, yielding sine and pulse in a stochastic ratio very close to 50%. Then, at progressively higher levels of female movement, the signal biasing production towards one type becomes stronger, up to the point where there is a clearly unequal balance of the two songs. Specifically, this signal is biased towards pulse production. Ostensibly, the state of the production system at any given time during courtship exists along some continuum where at one end all song is produced from the “indiscriminant” signal and at the other all song is produced from the “pure-pulse” signal. Importantly, the data strongly demonstrates that in wild-type flies the particular “state” within this continuum at any given time is a function of the target female’s movement.

When considering the implications of the second model, data pertaining to production of individual song types makes much more sense. Wild-type flies courting females who are moving relatively slowly produce pulse and sine song in nearly equal ratios (Fig 5A). The difference in production levels between the two song types does not become statistically different until approximately 4 crossings/window, after which point the difference is significant throughout. The close harmony of pulse and sine levels at these low levels of female movement resembles the production regime in which the indiscriminant signal dominates. The behavior of the production curves with
progressively increasing female movement can be thought to reflect the gradual shift from the indiscriminant signal towards the pure-pulse signal.

Among wild-type flies, increases in female movement beyond approximately 4 crossings/window both increase pulse production and begin to decrease sine production. Given the predictions of the model, one would expect increased movement to yield more pulse song but not necessarily less sine song. The fact that this simultaneous decrease is observed offers another important insight into the production mechanism: both of the two putative central command signals influence each type of song. This offers an explanation as to why the production curves of mutant flies are so dramatically different from not only one another but from wild-type as well. For nearly every level of female movement, Or83+ flies produce significantly more pulse song than sine song (Fig 5B) whereas Or83b^2 flies don’t produce significantly more pulse than sine until very high levels of female movement (Fig 5C). With the model in mind, this suggests that the different sensory deficits of these males affect their courtship strategies by influencing the way in which female movement affects the balance of the indiscriminant and pure-pulse signals. As can easily be seen, disrupting this balance has profound consequences on the sensory-motor transformation underlying courtship strategy.

3.4 Distance and Song Production

Upon engaging in courtship, the male commits across many dimensions of his own behavior. Indeed, the male is hardly stationary while singing and instead quite actively chases the female. As such, courtship tends to involve considerable proximity between the male and the female. Intuitively, if the male is trying to approach the female during courtship, his goal is made more difficult when the female is moving quickly. That
is, the distance between the male and the female is likely to be proportional to her level of movement. For a project investigating the role of sensory input in courtship strategy, distance is a key variable. In Section 1.3, I discussed how female movement was a logical context in which to examine courtship strategy, as changes in movement can influence the set of sensory cues the male receives. Such reasoning was based on the host of literature defining vision, gustation, and olfaction as important modalities for courtship (see Section 1.4) and the logical assumption that differences in female movement directly affect how the male would perceive his target’s velocity and, by influencing distance, indirectly affect the gustatory and olfactory profile of information he is receiving. The data presented in Section 3.1 suggest that distance might also play a role in visual processing. That is, assuming the Or83+ flies have restricted visual acuity, their courtship towards other males is perhaps a function of their decreased ability to distinguish conspecific males and females through visual information. Regardless of visual ability, a male’s proximity to his target should increase the ease with which he makes this discrimination, thus making distance a speculative yet intuitive dimension along which visual information impacts both courtship and courtship strategy.

In order to investigate the effects of distance on song production and, ultimately, courtship strategy, an object-tracking program was used to measure the distance between the two flies at each frame of the video captured while recording (see Section 2.4 for details). The average of each distance trace spanning 5 seconds before to 3 seconds after the onset of each bout of a given type of song was measured as an indication of the ideal distance stimulus for that type of song within a specific strain; this method of correlating distance and song onset is functionally a bout-triggered average. The results of this
analysis for wild-type flies are shown in Figure 6. The traces representing the ideal distance stimulus for sine are plotted in red while those for pulse are plotted in blue. One can clearly see that in all cases, less distance between the male and female is more conducive to sine production. Furthermore, the ideal stimulus for sine production seems to be a brief decrease in the distance separating the two flies. Thus, the probability of sine being produced is inversely proportional to the distance between the two flies. This is consistent with the way female movement and distance were predicted to interact in the generation of courtship strategy.

As discussed above, data supports a model in which central control produces sine only stochastically through an indiscriminant signal. However, pulse is produced by either the indiscriminant signal or the pure-pulse signal. Thus, while the distance average for sine should be representative of the ideal distance stimulus for eliciting or strengthening the indiscriminant signal, an important manipulation has been made to allow visualization of the ideal distance stimulus for eliciting or strengthening the pure-pulse signal. Figure 7 depicts the ideal
distance traces for all flies as well as the theoretical ideal distance trace for the pure-pulse signal, which is shown in green. These lines were obtained by adding the difference between the ideal-pulse trace and ideal-sine trace to the ideal-pulse trace. In other words, the blue curve represents the average between the red and green curves at any given time. The green curve should not be considered an exact representation of the ideal distance stimulus for the pure-pulse signal since it requires the assumption that throughout the experiment the indiscriminat and pure-pulse signals were equally active. This assumption can't be verified and is not likely to be true anyway, which is why the green lines can only be considered approximations. Regardless, they clearly indicate that the pure-pulse signal is strengthened following an increase in the distance separating the male and female. This data strongly supports the conclusion that increases in distance strengthen the pure-pulse signal whereas decreases in distance strengthen the indiscriminat signal. Such a conclusion fits nicely into the predictions made in Section 1.
and again at the start of this section that distance, as a function of female movement, is a key variable in courtship strategy.

The ideal distance traces of the wild-type and Or83b² flies offer some insight into the way olfactory deficits affect courtship strategy. When looking closely, one can see that the ideal distance traces are remarkably similar in shape between the two groups (Fig 7A and 7C). The only obvious difference seems to be that the traces are shifted upwards for the Or83b² flies. Such a fact suggests that, without olfactory input, the indiscriminant signals at any given distance are stronger in the Or83b² males than in the wild-type males at the same distance. This strongly agrees with the total production data of the Or83b² flies (Fig 4), as a ubiquitous increase in the strength of the indiscriminant signal should yield a proportional increase in raw song production.

Similarly, the ideal distance trace for the Or83+ flies can speak to the effect of this strain’s deficits on song production. As one can see, the characteristic upward inflection of the ideal traces for the pure-pulse signal (green curves in Fig 7) before the onset of a song bout is somewhat visible in the ideal-pulse trace of these males. Theoretically, if all pulse production occurred through activation of the pure-pulse signal, the ideal pulse trace would exactly mirror the green curve, whereas if all pulse production occurred through the activation of the indiscriminant signal, the ideal pulse trace would exactly mirror the ideal sine trace (the red curve). Presumably, that the ideal pulse curve of the Or83+ males displays the aforementioned upward inflection points to the fact that, among these males, more pulse song occurs as a consequence of the pure-pulse signal than of the indiscriminant signal. A shifted balance of the indiscriminant and pure-pulse signals from the brains of these flies could begin to explain why the Or83+ flies seem
more biased in general towards the production of pulse song in their courtship strategy compared to wild-type flies (Figs 2 and 3).

3.5 The Role of Sensory Inputs in Courtship Strategy

At this point in the project, the best way to validate ideas of how sensory information is used to generate courtship strategy was through the use of a computer model. The first step in doing this was to define the way in which saliency of a particular modality is related to female movement. In many ways, this required logical assumptions based on the known steps of courtship. Gustatory information is known to be transmitted through direct contact and is thus a function of movement in so far as distance is a function of movement. Intuitively, the saliency of gustatory signals is inversely proportional to distance. All males are modeled to have identical gustatory capabilities. Since it is not crucial to an understanding of the model’s basic workings, I will forgo describing the exact equations by which saliency of a given modality is a function of movement. Vision, though a single modality, likely impacts courtship in two ways. In the first, most straightforward way, the male is able to detect the motion of his target and integrate her velocity, making saliency a direct function of movement. In the second, the male’s proximity to his target increases his ability to identify her as a suitable target and

![Figure 8. Modeled Modality Saliency as a Function of Female Movement. Approximate saliencies of gustation (blue), vision_d (red), olfaction (yellow), and vision_m (green) are shown as a function of female movement in a noiseless system. The curves behave somewhat differently in the actual model.](image)
likely also provides an excitatory cue. Thus, two separate cues exist a function of vision, one is a direct function of movement and one is a direct function of distance and therefore an indirect function of movement. The third and final modality is olfaction. Since the substrates of ORNs are pheromones, there is likely a component of distance in the saliency of olfactory cues. At the same time, however, olfactory-specific pheromones are thought to be quite volatile, which should partially reduce the close-distance requirement and add a component of movement to olfactory salience. That is, higher female movement levels lead to greater pheromone dispersal, and thus increases olfactory salience. This reasoning is substantiated by the observation that Or83b⁵ courtship strategy tends to be most different from that of wild-type under conditions of high female movement (Fig 3).

At this point, saliencies of different modalities have been described as functions of movement, distance, or both. However, since distance can be thought of as a function of movement, all saliencies can be reconciled as functions of movement. Figure 8 depicts the theoretical saliency curves of each sensory input as a function of distance for wild-type flies. In this generalization, distance is more of a function of movement than is accurate, since distance is really only influenced by movement. Still, however, this figure offers a useful depiction of how movement is a variable across which all of these saliencies vary dynamically. Furthermore, saliency curves are updated according to their time constants. This is important for gustation and vision, which are modeled to have different time constants depending on whether the saliency is increasing or decreasing. That is, the saliencies of these inputs decrease slower than they increase. This mechanism was added to model the notion that these two sources of information serve to identify the
target, both as *D. melanogaster* and as a female. Presumably, these identifications are relatively stable once they are made and should not evaporate quickly due to a relatively brief increase in the distance separating the flies.

With saliency curves defined, the task becomes understanding how they interact to produce courtship strategy. Ideal distance curves (Fig 7) show that the indiscriminant signal (which alone presumably yields sine and pulse production in nearly equal ratios) is strengthened when the distance between the two flies decreases and that the pure-pulse signal is strengthened when the distance increases. Thus, modalities whose saliencies are inversely proportional to movement (gustation and vision\(_d\)) are probably those underlying activation of the indiscriminant signal while those directly proportional to movement (olfaction and vision\(_m\)) are probably those underlying activation of the pure-pulse signal. The fact that four different sensory mechanisms are responsible for the behavior of two central signals requires the involvement of sensory integration areas in the fly CNS. Putative integration sites have been already been identified (see Section 1), making the speculation that sensory integration plays a role in this model valid. Furthermore, it seems likely that the sensory integration sites interact.
That is, Or83b^2 flies eventually leave the equal-sine-pulse production regime at very high levels of female movement (Fig 3), inviting the speculation that the combined input of olfaction and vision_m must overcome a threshold in order to begin biasing the system towards pulse song. Since olfactory information is unavailable to these flies, this threshold must be crossed entirely by activity in the vision_m processing area. Only at high levels of female movement is this condition met. A likely source of this threshold is inhibition from the site integrating gustation and vision_d. The final layout of this model is depicted in Figure 9.

Figure 10 shows the output generated from the model in response to a somewhat randomly generated movement input and a pseudo-random distance input (that is, distance is modeled as a random process that is heavily influenced by movement). As one can clearly see, the model does an excellent job of recapitulating the courtship strategy data for each type of fly. The most impressive aspect of the model is that any differences associated with different fly strains are modeled entirely as changes to the raw saliency of a given modality or its saliency-movement curve. That is, all changes took place along the first row of nodes in Figure 9. For example, the only difference between the wild-type simulation and the Or83b^2 simulation is that during the latter all olfactory input was multiplied by 0. This manipulation is entirely analogous to the sensory deficits conferred by deletion of the Or83b gene in these flies. The Or83+ data could not be recapitulated with such a simple manipulation, but the nature of the visual deficits within these flies is not so simple either. Still, though, the difference between modeled Or83+ flies and modeled wild-type flies is entirely restricted to visual processing and alters only how salient given visual information is at a given movement level. Thus, the success of this
model in recapitulating observed data by making changes entirely analogous to the putative deficits in question greatly strengthens the conclusions made previously regarding the dichotomous indiscriminant/purc-pulse production model and the notion that courtship strategy exists as a dynamic integration of movement-dependent sensory cues.

**Figure 10. Comparison of Modeled and Real Data.** Data from the multiple runs of the model (open circles) are compared against sliding window averages of actual data (bold lines) for wild-type (A), Or83+ (B), and Or83b7 (C) males. Actual data is scaled to fit the x-axis such that the median level of female movement throughout all experiments is roughly aligned with 50% max. That is, all actual data curves are scaled using the exact same calculation where 15 crossings/window is defined as 100% max. Each modeled data point shows the average movement and %P in a single 30-second window.
4. Discussion

4.1 Possible Sources of Error

In general, this project did not require the use of complex technology or terribly sensitive equipment nor did it involve many automated processes. As such, it seems safe to say that any potential situations in which sophisticated techniques might become a source of error were avoided. However, the simplicity of my experimental set up was, at times, a potential source of error. The lack of reliable song annotation software at the start of the project required that I score data by hand. However, since I was in charge of raising and isolating the flies, I was not able to blind myself from the genotype being examined when scoring an individual file. While it was not experimentally ideal to risk the possibility that in ambiguous cases my decision to score certain sounds as pulse, sinc, or silence was subject to bias or expectations, the data closely match that obtained through automation once the program was available.

The use of crossings per window as an indication of female movement was perhaps another source of error brought about by overly simplified techniques. In order for this measurement to be perfectly accurate, the female must maintain a constant path throughout the experiment and a constant velocity during each window. The females did prefer paths along the perimeter of the chamber, but their velocities changed often. Thus two different windows labeled as having the same level of female movement could in fact label what are actually quite different movement patterns as the same. I am confident, however, that this error is accounted for through the number of repetitions, as the sliding window averages (which help to smooth out inconsistencies) display a strong dependence of courtship strategy on movement. Thus, inaccuracies in this measurement were probably not severe enough to occlude meaningful trends.
Other error may stem from the assumptions surrounding the visual capabilities of the Or83b² males. That is, since these males only barely courted other males when testing for homosexual courtship (Fig 1), and since this behavior was significantly less than that of Or83+ flies, the assumption was made that the extent of their visual deficits were not equal. Unfortunately, there is no way to test the limits of their visual capabilities and, even though it is highly unlikely that Or83b² have wild-type visual acuity, they are assumed to have functionally normal vision for the sake of courtship. To whatever extent this assumption is not valid, error arises in the interpretation of the data. While the results of the model (Fig 10) provide good evidence that the core of the Or83b² flies' deficits pertain to olfaction, I cannot rule out the possibility that some aspects of their behavior are a consequence of altered visual processing.

Assumptions regarding the visual capabilities of the Or83+ flies are also a potential source of error. While it is much more certain that these flies possess visual deficits, it is partially assumed that the consequences of ectopic mini-w expression are limited to sensory physiology. While there is literature describing the light-dependency of homosexual courtship among males misexpressing mini-w (Hing and Carlson, 1996), attempts to ensure that wild-type and Or83+ males were functionally similar in total darkness were non-conclusive. This was largely an issue of time. A recording setup was created that involved testing courtship of virgin CS females under only IR light (which the flies perceive as total darkness). While wild-type and Or83+ did court and Or83b² flies did not (data not shown), the total amount of courtship recorded was too little to generate any meaningful conclusions. With more time, the sheer volume of experiments could have been increased so as to allow a comparison between these groups of males.
Hopefully, such a comparison would have confirmed that differences between Or83+ and wild-type males were limited to capabilities of visual processing.

4.2 Roles of Pulse and Sine Song
The results presented above have considerable implications for the continued research of Drosophila melanogaster courtship behavior. The first that should be addressed are those relating to the roles of pulse and sine song during courtship. Specifically, these results offer strong insight into the elusive role of sine song. While the species-identifying role of pulse song is well-established, the question of sine song’s part in achieving copulation remains to be satisfactorily answered. Female receptivity is thought to be displayed by her movement level and the above data demonstrates that as a female slows down, the male phases more sinc song into his courtship. Since the two most obvious criteria by which a female must choose a mate are conspecificity and health, with the former being the more absolute requirement, pulse song and sinc song perhaps convey those two forms of information. According to this interpretation, high levels of female movement occur when a male whose species she has not yet confirmed is her own is courting her. This functionally signals to the male that he must provide said confirmation—hence, pulse song. As the female slows down she enters what can be considered a different stage of receptivity in which she requires less information pertaining to the male’s species and more pertaining to his health. Presumably, sinc song conveys that information.

Another possibility is perhaps that the female integrates her own receptivity/movement behavior into an expectation of how the male will behave in terms of pulse and sinc composition. The above data provides compelling evidence that deficits
in a male’s sensory processing can yield dramatic changes to his courtship strategy. If the female were sensitive to these changes, deviations in the male’s courtship strategy from her internal expectations could provide a signal of the male’s health along a much more subtle dimension. Indeed, there is almost certainly some sort of comparison on the part of the female between the male’s expected behavior and his actual behavior. That is, if each type of song increased female receptivity in a manner proportional to its total duration, Or83b² males would tend to copulate fastest. This is abundantly not the case. Throughout the experiments, females were very noticeably more receptive to wild-type males than to mutant males (data not shown). Assuming the only perceptible difference between these groups of males as far as the female is concerned is their ability to modulate %P according to her movement, this strongly argues that the female is concerned with the male’s courtship strategy.

There is no reason that these two interpretations are mutually exclusive. The information the female requires and the information she expects could very easily be the same at any given point. Furthermore, these two interpretations involve selection criteria that are not mutually exclusive. That is, in the first interpretation, the female uses the acoustic qualities of the male’s sine song to assess his health, and, in the second interpretation, the female uses the male’s courtship strategy to assess his ability to integrate sensory information. Whether the first interpretation, the second, or both is/are correct, the evidence is clear that song production must be put in the context of female behavior for a clear understanding of its function.

The failure of previous playback experiments to incorporate this context perhaps explains their inability to conclusively establish a role for sine song (RYBAK et al. 2002a,
(ROBERTSON 1982). With this requirement in mind, however, it is perhaps now worth revisiting this technique but doing so in a way that recapitulates courtship strategy. This would offer a means by which to test the predictions of my interpretations. Specifically, by adjusting the extent to which the playback song recapitulates wild-type strategy, one could directly examine the extent to which song efficacy is a function of its placement in the context of female behavior.

4.3 Courtship Strategy as a Behavioral Model

The results presented here provide the first, to my knowledge, demonstration of courtship strategy. While this finding has significant implications regarding the roles of pulse song and sine song (as described above) it is also profoundly significant in an even broader sense. With Drosophila serving as an extremely useful and ubiquitous tool for research in genetics, the courtship behavior of D. melanogaster males has become a very popular model for unraveling the genetic and biological underpinnings of behavior. Indeed, one of the reasons it has served as such a useful model is the general innateness of the system. These results demonstrate that the system is not only innate but impressively flexible among wild-type flies and also quite sensitive to mutations affecting sensory availability.

Recently, the courtship behavior of male fruit flies was proposed as a promising model for the biology of decision-making (DICKSON 2008). Indeed, courtship strategy offers an excellent feature of Drosophila courtship behavior for bringing the biology of decision-making to light. Changing sensory cues require the male to use available information to form a judgment regarding the receptivity of his target and to update his behavior accordingly. Therefore, courtship strategy and its dependence on female
locomotion offer a direct link between sensory input and behavioral output. While the model described in Section 3.5 is able to recapitulate courtship strategy as a function of movement in a way that seems to capture the manner in which different sensory modalities are used, there is still much elaboration left to do. The circuit responsible for this particular sensory-motor transformation task is almost certainly more complex than the model itself. Characterizing the intricacies of the system beyond what is presented here should be a goal of future research.

4.4 Comments on Sensory Integration

When examining the data, one can see that there is a clear requirement for the male to consolidate several forms of sensory information into a decision. This process requires sensory integration and, as the model shows, likely interaction between central integrated areas. Exactly how the information is processed is key to courtship strategy, but this project did not focus on manipulating how such processing occurs. Rather, all manipulations had to do with what information the male had available to process. This was highly useful in creating a model that captures the logic behind this type of information processing, but the model remains only that. Perhaps the most exciting avenues opened through the discovery of courtship strategy are those pertaining to the actual neural computations involved in sensory integration during courtship. This system holds considerable promise as a model for the higher-order processing of a relatively lower-level organism.

In the wild or even in a crowded test tube, the courting male likely has to deal with competing sensory cues in order to faithfully direct courtship towards his target. To the extent that other male or female flies were around, the raw saliency of any particular
modality may cease to be a function of only the male's target. However, in order to maintain courtship strategy, the male would have to attend to only the profile of cues emanating from his target. This is likely among the roles of sensory integration. And, while this may be a considerable demand to place on a simple neural circuit, it seems a necessary capability for the system to have if courtship strategy is to be maintained in conditions where more flies than just the courting male and his target are present.

It is perhaps possible that the males lack the capability to ignore gustatory, olfactory, and visual information from non-target sources. However, the fact that courtship strategy is innate points to an evolutionary influence and it seems unlikely that the evolutionary process tailored courtship to function only under such ideal circumstances. As such, I favor the possibility that males are capable of ignoring competing sensory information. The above model (Section 3.5, Figs 9 and 10) of courtship strategy can offer some insight into what the mechanisms of attenuating irrelevant stimuli might be. While these claims require experimental validation before they can be considered anything but speculative, it seems feasible that sensory integration serves to compare the dynamically changing sensory cues to one another. Assuming—as I believe is entirely justified given the above data—that saliency of a given modality is a function either directly or indirectly of target movement, a given change in saliency of one modality should be predictive of a similar change for the other. Specifically, gustation and vision_a should be very predictive of one another as should be olfaction and vision_m. The key notion is that the site integrating two covarying modalities represents the sum of their activity. Thus, the inhibition imposed by the gustation/vision_a integrator onto the olfaction/vision_m integrator (Fig 9) creates the condition that any stimulus that
stimulates olfaction or vision\(_m\) alone (i.e. something that is not the target female) must be twice as "powerful" as a stimulus that stimulates olfaction and vision\(_m\) proportionally (i.e. the target female) to overcome the inhibition. As such, even in the very basic model presented here, there is a potential albeit rudimentary mechanism for ignoring stimuli less likely to come from non-target sources.

To the extent that this is an accurate interpretation of how certain aspects of the sensory information might be processed, this courtship strategy circuit can be considered responsible for making what is ultimately a probabilistic decision. Within the probabilistic framework, the innate system exhibited by these naïve flies utilizes inherent assumptions about how separate yet related signals will covary. As in the case of the mutant flies, when those assumptions are false courtship strategy deteriorates. Following an investigation into the extent, if any, to which the courtship strategy circuit is in fact making a probabilistic decision regarding the source of the sensory input, the next question one must ask is whether the circuit is plastic. When one of the assumptions that olfaction and vision\(_m\) covary and gustation and vision\(_d\) covary become false—as is the case for the Or83 flies—does the circuit adapt? In many ways, the question of adaptability is central to a probabilistic circuit. One might expect that if the system were truly working probabilistically it has the capability to extract the statistical relationship between two variables as determined by experience, and this is an avenue that is very much worth exploring.

4.5 Future Experiments
First and foremost, a necessary future experiment is one in which the courtship behavior of Or83+ is compared to that of wild-type males in total darkness. Preferably,
enough data would be collected to allow an examination of courtship strategy both as a function of female movement and distance. While this experiment is functionally a control, it would give more concrete meaning to the data presented here.

Another interesting future experiment would be to test how females respond in playback experiments in which the song is manipulated to reflect wild-type strategy to varying degrees. As described above, females were observed to prefer wild-type males, presumably because of their proper modulation of %P in response to the females’ movement. If playback experiments were able to recapitulate these preferences, it would add considerable weight to the argument that the roles of pulse and sine song are not static but instead depend on the context of the female’s behavior.

Lastly, future experiments should investigate how the courtship strategy of sensory-deficient males (such as Or83b\textsuperscript{2}) changes through continued opportunities to court female flies. Results demonstrating the ability of these males to learn proper courtship strategy—presumably by learning that olfaction and vision\textsubscript{m} will not covary—would be of incredible importance. Such would further demonstrate the usefulness of courtship strategy as a tractable model for studying the cellular mechanisms by which circuits adapt to changing statistical relationships between variables in the external world in a way that drives behavior.
5. References


