Drosophila Tone Preference During Oviposition

Alexa Christine Decker
Abstract

Evidence shows that *Drosophila melanogaster* take into account the environmental factors such as substance, temperature, texture, and direct threats in order to provide protection for their offspring; young drosophila utilize experienced drosophila's olfactory cues for informed site selection. Their site selection is indicative of what drosophila view as beneficial. Since they have near-field hearing, drosophila may integrate sound into their deciding factors. If drosophila utilize sound as a deciding factor then a tone within their hearing range and similar to the tone their wings produce during flight such as a 250 Hz tone could have positive or negative associations. In order to assess their tone preference and to confirm previous findings 120 female drosophila were collected. Their preferences were measured by counting the amount of eggs laid and categorizing the areas they were laid into zones of On, Near, and Off relative to tone location. There were two independent variables with three levels each. The first independent variable was tone presence; either the 250 Hz tone was present on one side of the forced choice chamber, present on both sides, or there was no tone. The second independent variable was substrate. The substrate consisted of sucrose a food source, caffeine a bitter avoided substance, or agar a neutral solution. They laid significantly more eggs under the tone when agar was underneath whereas they laid at random in the no tone control group. When given the option to lay their eggs under tone or on sucrose they chose tone at a significant rate over sucrose. This study demonstrates that young female drosophila may positively associate 250 Hz tone, and place more value on social feedback than their own assessment of resources.
MONTCLAIR STATE UNIVERSITY

/ Drosophila Tone Preference During Oviposition /

by

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DROSOPHILA TONE PREFERENCE

DROSOPHILA TONE PREFERENCE DURING OVIPOSITION

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The 2.5 millimeter long *Drosophila melanogaster*, commonly referred to as the fruit fly, has surprisingly complex cognitive capabilities. Cognition is normally interpreted as choices and decisions made from information processing in the central nervous systems of humans and nonhuman species (Griffin & Speck, 2004). Drosophila have approximately 100,000 neurons (Bellen et al., 2010), which they utilize for encoding, storing, and recalling information. They weigh the costs and benefits affiliated with the surrounding possibilities to decide the best course of action. Possibly the most intricate and interesting behavior they exhibit is oviposition discretion. Not only do they plan to perpetuate their own lives, but that of their offspring. Though several factors attributing to their discretion have been examined, auditory cues have not been explored.

Similarities exist between humans and drosophila despite considerable differences in taxology. Seventy-five percent of disease-causing genes in humans have a functional homolog in drosophila (Reiter et al., 2001; Lloyd & Taylor, 2010). Drosophila’s cognition is even similar to humans’ on the cellular level, both in the metabolic sense and in the pathways signals follow (Pandey, & Nichols, 2011). Drosophila’s metabolic system so closely resembles the mammal’s that drugs effect their central nervous system in a comparable manner (McClung & Hirsh, 1998; Moore et al., 1998; Bainton et al., 2000; Nichols et al., 2002; Rothenfluh & Heberlein, 2002; Satta et al., 2003; Wolf & Heberlein, 2003; Andretic et al., 2008). Parallels are also found on the behavioral level, most convincingly for circadian rhythms (Panda, Hogenesch, & Kay, 2002), sleep (Shaw et al., 2000; Shaw et al., 2002; Hendricks et al., 2000), and learning and memory (Bolger, et al., 1993).
The behavior and neurological function of drosophila during their period of inactivity closely resembles sleep in mammals (Shaw et al., 2000; Shaw et al., 2002; Hendricks et al., 2000). Drosophila will choose a specific location to sleep and will remain immobile for around two and a half hours mainly during the night. During their time of immobility they are effectively unresponsive to sensory information. Drosophila’s arousal thresholds were measured utilizing vibration, visual, and auditory stimuli (Shaw et al., 2000; Nitz et al., 2002; Huber et al., 2004). When a drosophila is awake they respond immediately to medium intensity stimuli. Drosophila that have been inactive for at least five minutes experience slower motor responses and will only arouse when stimuli increases in intensity. When drosophila are deprived of sleep they have difficulty learning, indicating sleep may be as important for attention and memory consolidation as it is in humans (Seugnet, et al., 2008).

Drosophila have the capacity for short-term and long-term memories, and demonstrate learning capabilities while fighting for limited resources in their natural environments. Typically the fights are in same sex pairings, and although the strategies utilized by the separate sexes vary some strategies are shared (Nilsen, Chan, Huber, & Kravitz, 2004). Female fights, regardless the winner, result in equal division of resources (Nilsen, Chan, Huber, & Kravitz, 2004). Both genders learn to modify their fighting strategy based on past encounters (Yurkovic, et al., 2006) allowing them the advantage in subsequent fights. Young male drosophila attempt to mate with members of different species and other males (Dhole & Pfennig, 2014). They eventually narrow their selection down to females in their own species based on their past experience. Males also learn to
modify their courtship strategy after sexual rejection (Ejima, et al., 2005; Ejima, et al., 2007). They alter their approach behaviors and assimilate successful maneuvers into their routine. Young females will utilize the olfactory cues of experienced females in order to learn optimal egg-laying sites (Battesti, Moreno, Joly, & Mery, 2012).

Due to their ability to alter their behavior based on past experience drosophila are susceptible to classical conditioning. Previous studies have employed olfactory associations to test drosophila’s adaptivity. Tully and Quinn (1985) used shock pulses and two different odors; one odor was consistently paired with a shock and the other was not paired with anything. The odor paired with shock elicited an avoidance response even twenty-four hours after it occurred (Tully & Quinn, 1985). Reward conditioning is also effective (Kim, Lee & Han, 2007). An odor was paired with sucrose creating a positive association between the unrelated odor and sucrose. Significant decreases in memory happened at thirty minutes, one hour, and finally three hours after training. After six to twelve hours there is little trace of the memory (Kim, Lee & Han, 2007). The neurotransmitters responsible for learning and memory in drosophila include acetylcholine, dopamine, GABA, and serotonin (Gu and O'Dowd, 2006; Liu, Krause, & Davis, 2007; Wu et al., 2007; Sitaraman et al., 2008; Waddell, 2010). Parallels to deficits in learning and memory on a genetic level have been found in the mutations GNAS (Connolly et al., 1996), NF1 (Guo et al., 2000), FLNA (Dubnau et al., 2003), RSK2 (Putz et al., 2004), FMRI (McBride et al., 2005), PRSS12 (Didelot et al., 2006), UBE3A (Wu et al., 2008), PQBP1 (Tamura et al., 2010), EHMT1 (Kramer et al., 2011), NSUN2 (Abbasi-Moheb et al., 2012), ANK3 (Iqbal et al., 2013), CEP89 (van Bon et al., 2013) and
Implementing the readily available, easy to care for, drosophila melanogaster as a simple model for decision making is tenable, but there are understandably large differences between drosophila and humans. For example, the fly does not have an adrenergic system nor do they have epinephrine or norepinephrine. Instead drosophila use octopamine. Octopamine is only a trace amine in humans, but in drosophila it is a major neurotransmitter with similar functions as the adrenergic neurotransmitter system of mammals (Evans & Maqueira, 2005). Neurotransmitters present in drosophila might not have the same variety that mammals have and may serve different purposes in regulating behavior (Nichols, 2006). Exploring animal communication, behavior, and physiological capabilities of various species may provide insight into how they evolved (Gerhardt & Huber, 2002). Examining the cognition of a similar yet less complex brain structure allows us the opportunity to reveal the necessary and sufficient conditions for meaningful neural circuitry and identify variations (Olsen, & Wilson, 2008), furthering our understanding of neuron structures.

Drosophila exhibit behaviors such as courtship, resource acquisition and retention, and grooming (Pandey, & Nichols, 2011). They can plan to avoid objects moving toward them. Originally their avoidance behavior was considered a reflex in the giant fiber system, however, Hammond and O'Shea, (2007) identified a pathway from the brain to the thorax which sends a message which directs the wings to elevate prior to take off. This shows that drosophila can plan to perpetuate their lives utilizing their central nervous system.
Drosophila’s perception helps them process and retain sensory information, allowing them to consider different factors when navigating their world. Visual images are stored through retinotopic matching. This retention allows them to learn which areas are safe and which are to be avoided (Cartwright, B. A. & Collett, T. S. 1983; Wolf R, Heisenberg M: 1991). They can even distinguish abstract visual symbols and recognize objects from different orientations (Heisenberg M., 1995; Tang S, et al., 2004; Liu G, et al., 2006). Not only are they able to create a 3-D representation of the object, but they recognize when the object is removed from their environment and where it was previously located (Neuser K, et al., 2008).

Drosophila are able to combine their visual information with olfactory information (Frye & Dickinson, 2004; Guo J., & Guo A., 2005; Chow & Frye, 2008; Duistermars & Frye, 2008), allowing greater foraging acuity. Since they are able to detect minute changes in odor concentration and utilize visual information they can approximate how much food is in an area efficiently (Louis, et al., 2008; Faucher, et al., 2006). Olfactory information can also provide social cues. Several different behaviors of drosophila rely on social cues; for example locomotor activity (Levine, Funes, Dowse, & Hall, 2010), aggregation (Wertheim, 2001), aggression (Wang, Dankert, Perona, & Anderson, 2008), avoidance (Suh, et al., 2004), feeding (Tinette, Zhang, & Robichon, 2004), reproductive behavior (Ferveur, 1997; Ferveur, 2005; Fujii, Krishnan, Hardin, & Amrein, 2007; Svetec, Houot, Ferveur, 2007), sleep (Ganguly-Fitzgerald, Donlea, & Shaw, 2006), and oviposition in certain females (Battesti, Moreno, Joly, & Mery, 2012). Not only do drosophila have a direct perception of their surroundings, but they are also
able to imitate what more experienced drosophila have done in their place increasing their chances of survival.

Drosophila have a well-defined auditory pathway (Boekhoff-Falk, 2005; Eberl, Boekhoff-Falk, 2007; Kamikouchi, 2013; Morely, Steinmann, Casas, Robert, 2012). Their auditory system consists of a Johnston’s hearing organ and antennal receptors. The antennal receptors can sense near-field sound, gravity, and wind (Boekhoff-Falk, 2005; Eberl, Boekhoff-Falk, 2007). Near-field sound is definitively detected because female drosophila need to be capable of hearing the courting male. Both males and females produce sound during their flight. Drosophila beat their wings 200 times per second (Altshuler, et al., 2005) which is similar to 250 Hz tone. Insects and anurans utilize auditory signals for communication. More often than not the communication is for mating. Drosophila engage in a mating ritual which involves two components. The male drosophila to vibrate his wings creating a sound similar to a sine tone and a pulse sound (Ewing, 1964; Shirangi, Stern, & Truman, 2013). The pulse sound has been found to increase female receptivity. It has a higher level of intensity than the sign tone. Females have a neural representation of the parameters of the species’ code, which is genetically determined, and they reject or accept the male depending on the outcome of the comparison of the actual song to the representation. Typically drosophila perceive sound between the ranges of 100 Hz to 300 Hz (Dickson, 2008). The male is typically standing about three millimeters from the potential mate. Their near-field perception is less sensitive to ambient noise (Murthy, 2010). The auditory pathway is similar to that of mammals (Kamikouchi, 2013) such that directionality is perceived with a degree of
accuracy (Morely, Steinmann, Casas, & Robert, 2012).

Given the relative complexity of their cognition, drosophila’s ability to discriminate between viable egg laying sites for the well-being of their offspring is more comprehensible. Drosophila have a specific procedure for egg laying (see appendix A Figure 1). They will search the area immediately post feeding, probe the environment with their proboscis and ovipositor prior to egg laying to evaluate the virility of a site, and then either accept or reject the medium (Yang et al., 2008). The search time varies from a few seconds to a few minutes (Yang et al., 2008). Drosophila reject sites by withholding their egg and continuing their search behavior (van Delden & Kamping, 1990; Takamura & Fuyama., 1980; Allemand & Bouletreau-Merle, 1989; Spradling, 1993; Richmond, Gerking, 1979; Eisses, 1997). Acceptance entails a bending at the abdomen into the substrate, then forward and backward motions for about six seconds (Yang et al., 2008). After the egg is deposited they clean themselves and rest. Drosophila’s life-stages take an average of ten days to complete while drosophila can live up to thirty days. Drosophila are fertile eight hours post emergence (Pitnick S, 1996). Egg laying is a costly decision, they can only lay one egg every oviposition, it utilizes energy and time therefore selecting their site carefully allows them to lay eggs more efficiently (Yang et al., 2008).

The behavior seems to be moderated by a distinct subset of dopaminergic neurons. Similar to what is called neural Darwinism in humans, drosophila’s neural systems compete to either enhance or inhibit egg-laying preference (Azanchi, Kaun, & Heberlein, 2013). A group of insulin-like peptide 7 producing neurons communicate
when to release the egg (Yang et al., 2008). Suitability is determined by several different factors despite the inclination to assert that drosophila deposit eggs in a binary fashion based on the presence or absence of food, although food is a large part of their consideration.

Typically drosophila prefer to lay eggs directly on sucrose as opposed to bitter or salty mediums (Amrein & Thorne, 2005; Chyb, Dahanukar, Wickens & Carlson, 2003; Dahanukar, Foster, van der Goes, van Naters & Carlson, 2001; Scott, Brady, Cravchik, Morozov, Rzhetsky, et al., 2001; Slone, Daniels & Amrein, 2007; Schwartz et al., 2012), however they will sometimes lay their eggs close to, but not directly on sucrose if the area is unusually small (14.5x18.4mM) (Yang et al., 2008). This preference may be due to an assessment of near future foraging costs for the larvae. If the perceived foraging costs for the emerging larvae are high, as in the larva would have to move far for sustenance, then the drosophila will reject any other site besides sucrose (Yang et al., 2008). When faced with food mixed with menthol and regular food, drosophila will lay eggs on both substances. However, when drosophila are only allowed to eat food mixed with menthol they avidly avoid ovipositioning on this substance. If drosophila are given a choice in consuming regular food and food mixed with menthol then there is a decreased aversion to menthol, even attraction in some flies (Abed-Vieillard, Cortot, Everaerts, & Ferveur, 2014). This behavior may be brought about in order to insure future generations will be able to adapt to their environment. If they are raised on menthol based foods and consistently lay on methanol food then they will lose their ability to consume diverse foods. When drosophila have varieties of food available there is greater genetic variance
thus an increased prosperity in future generations (Abed-Vieillard, Cortot, Everaerts, & Ferveur, 2014).

Although food largely influences female drosophila’s decisions, there are other factors they consider. Though drosophila can lay thousands of eggs within their lifetime, they must maintain site selectivity to reduce the costs associated with egg laying. Judging their oviposition site based on the presence or absence of food does not provide enough information about the potential well being of their offspring. Using factors such as edges (Schwartz et al., 2012), temperature, social cues, and presence of a threat leads to a more informed decision (Kannan, Reveendran, Dass, Manjunatha, & Sharma, 2012; Yang et al., 2008). Edges as opposed to flat surfaces may provide some protective element against wind and/or predators. Mid-range temperatures can also serve as protection for the eggs since extreme temperatures have deleterious effects on the physiology, ecology, and fitness of drosophila.

Though drosophila are found in most places across the world, extreme temperatures are still injurious and potentially deadly for them (Dillon, Wang, Garrity, & Huey, 2009). Since eggs are unable to avoid exposure due to their immobility for the first 24 hours (Huey et al., 2002) the female’s choice largely determines her offspring’s safety. Eggs can hatch in less than twenty four hours, and emergent larvae have the ability to thermo-regulate, but until that time period eggs are subjected to the environment they were laid in (Huey et al., 2002). Light may be an indirect indication of temperature cues therefore drosophila prefer laying eggs during the afternoon, when light is present, but temperatures have peaked.
The presence of a threat also alters egg laying behavior. When a site is infested
with wasps, drosophila will avoid using that area and choose a clean site instead
(Lefèvre, et al. 2011). Wasps are parasitic they lay their eggs within the egg and larvae of
drosophila. Drosophila will withhold their eggs to avoid the inevitable loss associated
with a wasp infestation. Had drosophila not developed their behavioral modification they
would have had to develop physiological defenses. Only the eggs who had strong enough
shells would survive thus they would give birth to strong shelled eggs and the population
could continue despite the infestation. Evolving physiologic defenses is a passive means
of defense; instead drosophila avoid, groom, and self-medicate to perpetuate the lives of
their offspring (Lefèvre, et al. 2011).

There are two possible outcomes if drosophila discriminate their egg laying site
utilizing auditory signals as a factor in their decision, either they find the tone
advantageous or dangerous. Tone could resemble several representations namely another
drosophila. If the tone is perceived as another female in the area then they may find it
advantageous. Typically the social cues of other females are transmitted via olfactory
signal. Since females typically share resources and younger drosophila use more
experienced female drosophila’s choices as guidance then they may move towards the
tone in hopes of receiving further cues. In general if another drosophila is present the
female may move toward the tone since the other’s presence may indicate resource
availability.

If drosophila are drawn to sound as a positive social cue then further research
could uncover organisms’ general attraction to sound. Several non-human species exhibit
musical capabilities (eg. songbirds, gibbons, and whales (Miller, 2000)). In songbirds
music seems directly related to their mating call, aggression, or staking out their territory
(Sacks, 2007). If more simple representations of sounds within their hearing range attract
drosophila and other musical animals, then their attraction may indicate that even beings
as fundamental as insects are musically inclined. Therefore the complexity of music
exhibited and enjoyed by humans comes from a more basic attraction to sound.

Among humans the singing voice provides greater volume which may have
facilitated group interactions-similar to the basic auditory social cue drosophila may
perceive. Associated with grooming and social bonding in humans, singing would allow
for about four people to “converse”. The advantage of having small bonded groups is
their ability to ward off predators, however the disadvantage of larger groups includes
conflicts of interest and general discord. Roederer (1984) made a modern day observation
relating to the ability of music to facilitate group bonding. He recognized the importance
of music in superstition, religion, and the military tradition. Music established behavioral
coherency, which is consistent with the idea that music instills empathy. Dunbar (2004)
indicated that music has the ability to synchronize mood. Synchronizing moods would
allow groups to create similar goals and work on the same problem providing more
approaches and thus allowing a greater likelihood for finding a solution. With a group
that is small enough to communicate to each other yet large enough to ward off enemies,
singing might have been an important bonding tool and an effective means of
communication (Huron, 2001).

Protest for the benefits of louder volumes comes from sound’s ability to attract
predators. If female drosophila perceive the tone as a male or a predator, then they may avoid it. They may avoid it if they recently mated since males provide resource competition. Female drosophila have a long period of refractory before remating, they typically will feed post copulation this adding to the cost of egg laying (Barnes, et al., 2008). Thirty to fifty percent of recently mated females will remate within six hours of the original copulation (Vanvianen & Bijlsma 1993); Eighty percent will remate within four hours, (Bretman & Chapman, 2008-unpublished data). The female is also at risk of the male taking the food source around the egg or engaging in a territory fight.

Drosophila are sedentary organisms, often spending time by themselves except to mate. Female drosophila would want to avoid the possibility of her offspring being killed by a male and thus avoid tones similar to their wing beats. However, the advantageous nature of seeking out an experienced female drosophila may prove greater than the risk of encountering a male.

The purpose of the current study was two-fold. First I wished to further test the notion that oviposition serves as an indicator of evolved preferences in drosophila. I expect to replicate the hypotheses that sucrose is a positive substrate and caffeine is a negative substrate. Discretion on the substrate level would reinforce the idea that drosophila are actively choosing their egg laying positions. Secondly, I wanted to determine how sound factors into their preferences. I believe that if sound is utilized as a deciding factor it could be either perceived as positive or negative. My first null hypothesis was that there will be no differences between the groups, that drosophila do not discriminate their egg laying site based on food preference. My second null
hypothesis was there will be no group differences based on the presence or absence of tone.

Methods

Subjects

There were 120 female drosophila melanogaster collected and randomly assigned to twelve conditions. There were ten females per condition. Drosophila who produced less than five eggs during the 48 hour egg laying session were replaced.

Husbandry

Drosophila were kept in standard 95 by 25 mm vials. The vials in which drosophila resided were prepared by mixing dehydrated starch with 13 ml of ionized H2O. Twenty yeast pellets were added to the top of the mixture along with plastic netting. Vials were kept in an incubator which had an average temperature of 27 degrees Celsius. The incubator has a twelve hour light/dark cycle.

In order to control for age, adults were eradicated from the vials. Only larva and pupa remained. The emerging adults were separated by sex every 24 hours insuring the youth and sexual vitality of the subjects.

Procedure

There were two independent variables with three levels each. The first egg laying environment was defined as 250 Hz tone, no tone over the substrate, or tone opposite of the substrate. Placing the speaker above the substrate was an effective way to evaluate drosophila sound preference since drosophila perceive directionality and near field sound. The second independent variable was the substrate, which was either sucrose,
caffeine, or agar. The dependent variable was egg laying preference which was
determined via egg count (see Appendix A Figure 2 for more information). Eggs were
categorized into On, Off, and Near zones relative to tone location. If the tone was playing
over the area the eggs were laid this was considered On, if the eggs were laid opposite of
the tone this was considered Off, and the center was considered Near. If tone was absent
or playing on both sides the substrate was sucrose, caffeine, or agar throughout the forced
choice chamber.

Female drosophila were placed in a freezer for four minutes to immobilize them.
While immobilized they were separated and placed in the middle of the forced choice
chambers. Drosophila were free to explore the forced choice chamber for a period of 48
hours to provide them enough time to lay a substantial amount of eggs regardless of
fertilization (drosophila will lay unfertilized eggs). Preference was measured via egg
count after the 48 hour period.

**Assay**

Two hundred and fifty Hz tone was played through headphones placed over the
substrate. Tone was on full volume emitted from a Samsung Note media player. The 250
Hz tone was spliced through five star headphone jacks. A hole was drilled into the top of
the petri dish and the speaker was adhered over the hole for a more direct sound effect.
Speakers were on both sides of the forced choice chambers regardless of condition. The
forced choice chambers were larger than the petri dishes used in Yang et al’s (2008)
study. A forced choice chamber is essentially two petri dishes with a connecting smaller
circle in the center (see Appendix B Figure 3 for more details). The substrate was directly
under the speakers which either consisted of sucrose, caffeine, or agar. The substrate was prepared by mixing an agarose solution, then adding either caffeine, sucrose or agar. Agarose solution was prepared by dissolving agarose in ionized H2O constantly stirred and heated in the microwave for thirty seconds at a time resulting in a 3% solution. The proportion of sucrose to caffeine was ten to one.

The substrate was poured into the forced choice chamber with a cardboard rectangle containing the solution to one side and then agarose was poured on the other, filling the Off and Near regions. Light was consistently on since eggs are laid mostly during the day hours; the area was kept around 30 Celsius.

Results

I first confirmed that drosophila prefer laying eggs in sucrose and avoid caffeine. In order to assess this I examined caffeine and sucrose regardless of the tone condition and confirmed that caffeine is a highly avoided substance. Drosophila laid the least amount when caffeine was present-60 eggs on average (SD=1.9), the most when sucrose was present at 133 eggs on average (SD=5.4 eggs), and 98 eggs on average (SD=2.7) for agar. During the agar no tone condition egg laying was evenly distributed (see Appendix C Figure 4). Drosophila laid an average of 15 eggs (SD=1.7 eggs) directly on caffeine which was 32 percent of their eggs whereas they laid an average of 54 eggs (SD= 6.5) directly on sucrose.

Then I wanted to see if tone had an effect on egg laying. Since drosophila laid the expected average on agar, the percentage of eggs for agar was used as the expected value for the Chi square. All egg counts were converted to percentages.
Drosophila tone preference was evident when the agar 250 Hz was examined. Drosophila laid significantly more eggs under the tone $\chi^2(2, N = 120) = 37, p < 0.001$ (see Appendix C Figure 5). Drosophila laid the most eggs during the tone same side sucrose condition in comparison to the agar and caffeine conditions $\chi^2(2, N = 120) = 98.5, p < 0.001$. As expected drosophila avoided caffeine, laying the least amount of eggs when present. However, they laid their eggs under tone despite caffeine's presence on the opposite side $\chi^2(2, N = 120) = 27.6, p < 0.001$. More surprisingly eggs were laid significantly more underneath the tone when sucrose was on the opposite side $\chi^2(2, N = 120) = 42.4, p < 0.001$. There were overall more eggs laid in the 250/250 Hz egg laying conditions with an egg count of 383 in comparison to 186 in the no tone condition $\chi^2(2, N = 120) = 68.2, p < 0.0001$.

There was a significant left headphone preference in the 250/250 Hz agar condition $\chi^2(2, N = 120) = 12, p < 0.0025$. During the caffeine 250/250 Hz condition drosophila displayed a significant left side preference again $\chi^2(2, N = 120) = 11, p < 0.004$. The trend was also within the sucrose condition, though it was not significant $\chi^2(2, N = 120) = 3.7, p < 0.1572$. The combination of these results shows that drosophila prefer to lay their eggs under tone.

**Discussion**

Drosophila's cognitive capabilities allow them to process information and make decisions based on past experience. Drosophila are in fact actively altering their egg laying behaviors based on environmental cues and genetics (Schwarz et al., 2012; Peabody, Pohl, Diao, Vreede, Sandstrom, et al., 2009). The selectivity is moderated
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partially by indirect cues about what the current environment will be like in the near future, since their eggs cannot move and need to feed almost immediately upon hatching (Huey et al., 2002). The selectivity drosophila implement allows greater insight into what drosophila are perceiving and responding to in their environment and also provides insight into a possible fundamental feature of organisms in general.

Their simple yet relatively complex cognition makes them an ideal model for basic decision making. Their preference is easily measured since eggs are evident at the site they select. Some cues their selection is based on include food in the surrounding area, temperature, texture, and threats (Schwartz et al., 2012; Kannan, Reveendran, Dass, Manjunatha, & Sharma, 2012; Yang et al., 2008). They try to avoid laying eggs on bitter substances such as caffeine and avoid sites infected with wasps. They lay at moderate temperatures and use light as an indirect indicator of future temperature. Edges may have protective elements as well. Temperature and light were controlled for, along with edges and threats.

This experiment confirmed avoidance behavior by the overall reduced egg laying on caffeine. Drosophila indeed withheld eggs when a site is not suitable and invested more time and energy into egg laying when conditions were ideal as seen in the sucrose tone condition. If drosophila were raised on caffeine infused food, they might have exercised a greater aversion for caffeine, however since drosophila were raised on corn starch and given a choice between caffeine and agar during egg laying they tended to lay some eggs on caffeine. This supports Abed-Vieillard, Cortot, Everaerts, & Ferveur (2014) hypothesis regarding drosophila raised on menthol infused food and the subsequent
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avoidance during oviposition. This behavior is potentially for genetic diversification. Female drosophila laid their eggs directly on sucrose supporting the Yang et al. (2008) hypothesis that substrate size matters. Had the medium been smaller drosophila may have avoided laying directly on sucrose.

As expected drosophila modified their egg-laying behavior according to tone. This study indicates that drosophila find tone beneficial. This was demonstrated both through the increased egg laying on the side of the tone in the agar condition and the heightened egg laying during the sucrose tone condition. Further support for tone preference comes from increased eggs under the tone when sucrose was on the opposite side. In this instance tone was valued over food. Future experiments could test whether or not audition in smaller areas changes tone preference. Further studies could examine how readily drosophila can be conditioned to negative tone associations and how long the effects carry over for egg laying.

Drosophila may find tones within their hearing range as an indicator that other drosophila are nearby and that there are resources or protective factors associated with the presence of other drosophila. Since young female drosophila were collected it's possible that there was a positive association due to their youth. Younger drosophila seek olfactory cues from experienced females since it is assumed experienced drosophila have found success (Battesti, Moreno, Joly, & Mery, 2012). Since fights for resources typically happen more between the same gendered drosophila it's possible that young female drosophila were willing to take the risk of male presence for the benefits of experienced females (Nilsen, Chan, Huber, & Kravitz, 2004). The consequences would not be so
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deleterious as to deter the female. A future research direction could examine olfactory
cues and audition to demonstrate which factor drosophila utilize more heavily as
indicators of a site's potential benefits.

Humans might have been drawn to sound by the same basic tone preference
drosophila experience. Insects have made mating calls throughout evolution, humans
might have utilized their calls to hunt them or access other food sources surrounding the
insects. When this tone preference was paired with better representational capabilities
humans could have expanded their auditory associations beyond the basic tone
preference.

Music is universal, seen across all human cultures (Serafine, 1988). Music, like
most of the arts, is shrouded in mystery due to it's lack of definition and practicality. For
our ancestors producing music or enjoying music did not provide any direct benefit such
as shelter or food, yet flutes have been uncovered tracing back to the Paleolithic Age 30
000 to 40 000 years ago (Turk, 1997; Thomas, H., et al., 2012). Since wood does not
typically fossilize it is possible there were wooden flutes before there were bone flutes,
and considering the complexity of fashioning a flute it is reasonable to assume other
instruments such as drums far predated flutes (Huron, 2001). The simplest method of
making music would have been utilizing the vocal cords allotted to our ancestors (Huron,
2001).

Singing is associated with group bonding and social coherence in humans.
Drosophila seem to also experience a social coherence associated with tone. Thus singing
possibly began as a fundamental means of communication. Support for this theory stems
from music’s ability to evoke basic emotions in the listener (Thayer et al., 1994). Music is the third choice for altering sad emotions, and for reducing nervousness, tension, or anxiety. Music was one of the top choices for invigorating energy (Thayer et al., 1994). Tempo and mode effects both arousal and mood (Husain, G., Thompson, W. F., & Schellenberg, 2002). Since music has the ability to change the listener’s emotional state so readily, it is possible that the singer or producer of the sound is effectively conveying simple emotions. The listener is able to empathize with the singer thus understand the singer’s emotion.

Further evidence for music as a fundamental means of communication is motherese - the calming sounds mothers make to their children. There are elements of prosodic and differences in stress, pitch, volume, and emphasis within this system (Dissanayake, 1988). Motherese lends itself to a melodic voice and allows for simple emotions to be exchanged. Finally the singing voice carries farther than the speaking voice. Therefore when exploring unknown territories leaders might have found the singing voice beneficial. With the ability to communicate messages across greater distances humans could spread themselves farther out finding easier paths and more resources. The general draw toward tone could have aided the expansion of this capability. Similar to humans tone may represent another drosophila and the female utilizes the other drosophila’s tone as a measure of nearby resources thus drosophila also use tone for social coherency and resource acquisition (Battesti, Moreno, Joly, & Mery, 2012).

However sound still has the capability of attracting enemies. Older females may
avidly avoid the tone since they already have egg laying experience and do not need guidance from other females (Battesti, Moreno, Joly, & Mery, 2012). They would also want to avoid a potential threatening male since the benefits in this case would not outweigh the costs. More research should be conducted exploring age as a factor of tone preference. A briefer egg laying period with more fertile drosophila would also be a future research interest. Drosophila's priorities may be determined through reduced time.

There may also be a preference of volume considering the 250/250 Hz condition more accurate volume control could pinpoint the volume drosophila prefer. Headphones were placed at random relative to the testing room. The difference may be due to one set of headsets playing the tone louder than the other since the auditory signal was channeled through a 5 star headphone jack, therefore volume was not always consistent. Drosophila are able to distinguish between tones since 250 Hz received approach behavior and drosophila have a hearing range between 100 Hz and 300 Hz (Dickson, 2008) future experiments could compare the amount of egg laid relevant to different gradients of tone.

Given the ease of examining genetics in drosophila further research could be directed toward discovering the underlying genes responsible for tone preference. Since drosophila have basic characteristics in common with human they may be a model for understanding music preference in humans.
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Appendix A
Egg laying Drosophila

Figure A1: Yang et al., (2008) captured drosophila's egg laying procedure. First drosophila probe their potential egg laying site both with their ovipositor (far left) and their proboscis. They then oviposit and move forward and back (far right).

Figure A2: Image by E. Fast and H. Fryman: Drosophila eggs are visible immediately post oviposition with a magnifying glass.
Appendix B

Apparatus

*Figure B3:* Forced choice chambers were used with headphones adhered to the tops of each side. A five star headphone jack was utilized to distribute sound.
Appendix C

Results

Agar No Tone Condition:
Egg count for On, Near and Off zones

Figure C4: Agar no tone condition shows the egg counts for the zones. On in this instance is defined by the left headphone since there was no tone present. The distribution was as expected with the most amount of eggs laid in the largest areas in almost equal distribution.
Figure C5: 250 Hz tone was played on one side over agar. The largest amount of eggs were laid under the tone in the On zone.