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4 December 2015

Eye size in *Drosophila melanogaster* and how it affects peripheral motion vision

Abstract

Drosophila melanogaster, the fruit fly, is a holometabolous insect that requires a set amount of nutrients to induce metamorphosis; this specific point is called the critical weight. Once the critical weight is achieved, any further nutrients consumed during the larval stage are used to increase the size of the fly in the adult stage (Mirth, Truman, & Riddiford, 2005). One point to note is that as the body size of the fly increases, the size of the eyes that it can support also increases. As the size of the eyes increase, the number of ommatidia within the eyes increases, as well, allowing for more spatial acuity. The increase in spatial acuity can be seen in the frontal regions of the fruit fly's eye; however, it raises the question of how larger eyes and more ommatidia affect the peripheral vision. In many other insects, the peripheries of the eyes are used for temporal acuity, or to perceive motion. The hypothesis is that as the fruit flies' eyes become larger, their temporal acuity will increase.

Background Review

One primary source that was used was "Visual Acuity in Insects" by Michael F. Land. It includes information on the way insects perceive light and what the different factors are that affect their vision. One such factor is the interommatidial angles, which are the angles that separate the ommatidia in an insect's eyes. As the size of the angles decrease, the distance from which the insect can visualize an object increases. Therefore, as the size of the eye increases, the number of ommatidia within the eye should also increase, thereby bunching the ommatidia together into tighter spaces, which then also decrease the interommatidial angles. This decrease leads to a higher spatial acuity in the frontal regions of the insect's eyes; however, it is thought that the periphery will have larger ommatidia to better perceive movement. In the article, Land discusses that as flying insects near objects, such as vegetation, the motion blur they perceive in their periphery increases. This leads us to believe that if the frequency of the visual acuity within the virtual arena is increased, the flies will fly faster in the opposite direction. Therefore, it is imperative to keep the stimulus the same throughout the experiment (Land, 1997).

In addition, another article highlighted some potential obstacles in our research, primarily a mutation in *Drosophila* that hinders the sensitivity of peripheral photoreceptors. Called the *ninaA*^{P228} mutant, it decreases the concentration of rhodopsin within the fruit fly, affecting the R1-6 photoreceptors found in the periphery of the eye. This mutation is slightly different from a vitamin A deficiency in that it only affects R1-6 photoreceptors, while a vitamin deficiency affects many other areas of photoreceptors. The results of the experiment showed that when the control group of fruit flies were subject to a blue light near their periphery, they exhibited a prolonged depolarizing afterpotential (PDA); however, flies that were on a vitamin A deficient diet or had the *ninaA*^{P228} mutation did not show a PDA. Therefore, one important factor in this research that could potentially skew the data would be fruit flies with the mutated gene or a general lack of vitamin A to produce enough rhodopsin (Larrivee, Conrad, Stephenson, & Pak, 1981).

The article titled "The first steps in *Drosophila* motion detection" was also used in order to better understand the major mechanisms fruit flies employ to perceive movement. The article highlights the importance of having motion detection along with spatial acuity, since it is the foundation of flight for flies, as well as allowing them to find food and mates, and escape predators. It also discusses the method by which the flies perceive motion: Their retinas each have over 800 ommatidia, with each ommatidia having six outer and two inner photoreceptors. The outer photoreceptors are used to detect brightness, orientation, and motion, while the inner photoreceptors are used for color vision. Therefore, if the ommatidia at the periphery of the eye increase in size the outer photoreceptors would also become larger, which would allow for increased motion detection. While it is another possibility that the ommatidia in the periphery become more numerous rather than larger, it is more likely that they increase in size, since larger ommatidia allow for more temporal acuity. In addition to this, *Drosophila* detect motion using both direction and speed by delaying the signal of one ommatidia, while allowing an adjacent one to continue forward. This creates a separation of the two signals, which allows the system to compare the two signals and identify both the direction and speed of the motion. Therefore, it is not completely necessary to have many ommatidia in the periphery since just two of them would perform the task of determining direction and speed, which strengthens the hypothesis that larger fruit fly eyes would mean larger ommatidia in the periphery (Vogt & Desplan, 2007).

While many of these papers focus on how insects see and perceive motion, it was necessary to find an article that pertains to body size and eye size. In one experiment, researchers focused on the body size and eye size of *Chrysomya megacephala*, otherwise known as the oriental latrine fly. They hypothesized that the males with larger bodies would also have larger eyes, allowing them to be more active in low light conditions. Having larger eyes would mean that they required less light to see just as well as males with smaller eyes. The results showed just that: In four different populations, both larger males and females had larger eyes than smaller males and females. Larger males also showed more activity during the morning hours, but did not continue their activity into the evening. This furthers the hypothesis that body size and eye size are related in flies, which can be tested for verification in *Drosophila* (Smith, Palermo, Theobald, & Wells, 2015).

Delving further into the method by which to test the hypothesis that *D. melanogaster* will also have a positive linear correlation between body size and eye size, we look at another article that focuses on how fruit flies undergo metamorphosis. During their larval stage, fruit flies will consume as much food as possible and undergo molting, otherwise known as instars. It is at this time that the larvae will determine if certain milestones have been met. For example, between the second and third instar, the larva will use its size to determine if in the next molting it will remain a larva or undergo metamorphosis. In the last larval stage, it will determine if it has enough nutrients to sustain itself through metamorphosis due to starvation. It is only when the larva has successfully passed all the previous milestones that it will require a species-specific critical weight to induce metamorphosis. As soon as the critical weight is reached, juvenile hormone titers begin to decrease, which causes the release of prothoracicotropic hormones and the onset of metamorphosis. In order to test this hypothesis, researchers starved a fruit fly larva before it could achieve its critical weight. It was then re-fed and underwent metamorphosis. Since the larva was starved before the critical weight was reached, it could not undergo metamorphosis. Once the larvae were past the critical weight, starvation had no effect on when metamorphosis occurred. This brings up an issue in the methods that will be used in the experiment: It will be almost impossible to raise larger fruit flies without the use of hormones.

Since fruit fly larvae undergo metamorphosis soon after they reach the critical weight, they cannot be restrained in the food source and forced to continue eating. The use of hormones is also likely to skew the final data and become a factor in unreliable results. Therefore, in the experiment, the researchers will remove some larvae as soon as they begin to crawl out of the food source, which is known as their “wandering stage.” Other larvae will be allowed to remain in the food source for longer, even during the wandering stage. This will assure that some of the fruit flies will be smaller than the average (Mirth et al., 2005).

While this research project does focus on the peripheral vision of fruit flies, it uses both lab-bred flied as well as wild-type flies. This distinction is important in that lab flies are subject to differences that are in the control of the researcher, such as which larvae are allowed to eat more and mature sooner. However, in nature there are many constraints that cannot be completely replicated in a lab setting. One such difference is the number of stress factors that affect when the larvae fall off of their food source. Instead of removing themselves from the food sources as soon as they reach their critical weight, some larvae are forced to escape due to scavengers or other organisms eating the food that they are growing on. In order to avoid being eaten, fruit fly larvae will escape from their food source and continue their growth at a later period of time. While this sort of condition is difficult to reproduce in a lab environment, a similar experiment was done to test how fruit flies develop in nutritional stress. One group was reared in a standard medium of dry yeast, semolina, sugar, agar, ground raisins and water, while another group was reared on a poor medium with less dry yeast and no semolina or ground raisins. Once the adults emerged, they were sexed and measurements of their thorax length, wing length, sternopleural chaeta number, abdominal chaeta number, and arista branch number were taken. The results showed that flies reared in the standard medium had greater measurements in all five areas when compared to the flies from the poor medium. While the experiment does not account for predation, it does show that nutritional stress can cause a decrease in adult *D. melanogaster* size. Therefore, it can be hypothesized that the wild-type fruit flies in the experiment will have smaller body sizes and eye sizes (Imasheva, Bosenko, & Bubli, 1999).

While there is a significant lack of literature specifically on the peripheral vision of fruit flies, one experiment determined how flies develop a pattern for flight. In fruit flies, they fly in straight lines with occasional turns interspersed in the pattern. However, with many organisms, the path that they take is based on visual cues within their environment. By inducing a visual stimulus in the fly, the researchers were able to map out a pattern of movement, which mainly consisted of straight, forward motions mixed with saccades, or jerks to avoid collisions. This experiment indicates that fruit flies do indeed alter their flight patterns in relation to their surroundings, but more importantly, can alter their movements mid flight, meaning that they can also perceive motions using temporal acuity to determine if an object is growing closer. However, the experiment did not define which part of the fruit flies' eyes actually accounted for these motion detections, leading into our experiment (Tammero & Dickinson, 2002).

Many of the articles used have focused on visual acuity and growth patterns in insects. However, there has been little information on peripheral vision in *D. melanogaster*, which could possibly mean that there is a significant lack of research on this topic. It is understood that smaller interommatidial angles will allow for higher spatial acuity, there is a lack of surety of how this affects temporal acuity, which is what is used to detect motion. By allowing for higher motion detection, fruit flies with larger eyes will gain a significant advantage over those with smaller eyes because it will allow these flies to detect predators and potential mates faster.

Methods and Materials

In the experiment, both lab-raised and wild-type fruit flies will be used, and only the females from both groups will be tested. They will all be fed to their critical weight, while some will be removed from their food source earlier to induce a smaller adult size. The flies that are allowed to eat as much as they want until they proceed to pupate will be the control groups, while the flies removed from their food source prior to pupating will be the treatment group; both the lab-raised and wild-type flies will have one control group and one treatment group. Once they have fully matured, the female flies will be placed in a virtual reality arena where they will be subject to a visual stimulus near their peripheral view. This visual stimulus will take the form of concentric circles expanding from one side of the fly's peripheral view to shrinking on the other side. This pattern forms the illusion that the fly is nearing the expanding circles, which will cause it to move in the opposite direction, so as to avoid smashing into the wall it perceives is coming closer. In order to accurately test this, the flies' wing beats for each wing will be measured, specifically the amplitude of the wing beats. The amplitudes denote the number of times the fly beats that one specific wing, which gives us an indicator of what direction the fly is trying to move in.

In order to complete this experiment, several materials are required. To elaborate, several glass bottles with sponge caps are necessary to house the adult fruit flies. Some fruits will also be necessary to attract wild flies from the Florida International University Modesto A. Maidique campus. A mixture of fruits, yeast, and water will be used to raise the fruit flies and allow them to breed. Once several larvae are present within a glass bottle, the adult fruit flies will be moved to a new bottle so as to populate it. The larvae will grow on the fruit/yeast mixture for approximately three days. Once they have matured, they will be anesthetized by freezing and then separated into males and females based on morphology using a microscope. The females are then tethered onto a rod and placed under a microscope next to a metric scale and photographed. This scale will determine the eye and body size of the fruit fly. After the measurements are complete, the fly will be placed in the virtual arena and subject to the concentric circles stimulus. While in the virtual arena, their wing beats per second will be recorded. Once the tests are complete, the data will be compiled and the readings of the smaller fruit flies will be compared to the readings of the larger fruit flies. We hypothesize that the larger fruit flies will beat their wings faster than the smaller fruit flies because their larger peripheral ommatidia will allow for greater motion detection. In total, the experiment will require: Several glass bottles, fruits, yeast, water, two microscopes, several tungsten rods, adhesive, a metric scale, a virtual arena, a computer to form the code for the stimulus, and an apparatus to measure the wing beats.

While the amount of materials necessary seems difficult to acquire, some of these materials can already be found in Dr. Jamie Theobald's lab, which will be the setting in which the experiment will take place. Other materials that are either not found in the lab or not accounted for in this draft proposal will be bought using various research scholarships.

Discussion

While *D. melanogaster* has become one of the most common subjects used in experiments, there is a lack of literature detailing the vision of fruit flies. This may be due to their small size making it difficult to visualize their eyes directly or an overall lack of interest in the topic itself. However, by identifying the temporal acuity of fruit flies' peripheral vision, we can begin to determine how flies perceive motions around them and respond. This, in turn, opens up new opportunities to test other insects, particularly fliers, and observe how they respond to the same visual stimulus.

Conclusion

The final results of the experiment are expected to show that the body size of *D. melanogaster* and its eye size are positively correlated. Therefore, the larger the size of the fly, the greater its temporal acuity will be. This will be shown in the experiment where the larger flies will have a higher wing beat per second as compared to the smaller flies. While this could be due to their larger body mass, their wingspan should have also increased in relation to their body, which would rule out that possibility. The goal of this research is to establish a foundation for the peripheral vision of fruit flies in tandem with their body mass. Since little to no research has been done on peripheral vision of fruit flies, this research will be the beginning of a new set of discussions on how insects perceive motion.

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