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Innate Magnetic Directional Preference in *Drosophila melanogaster*

By

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An Honors Thesis Submitted in Partial Fulfillment
of the Requirements for Graduation from the
Western Oregon University Honors Program

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Abstract

The fruit fly, *Drosophila melanogaster*, is commonly used to understand genetic and behavioral mechanisms. This study is testing whether *Drosophila* have an innate directional magnetic preference based on the Earth's ambient magnetic field. The flies were tested using a sequential Y-maze that was housed within a Faraday cage to block out any radio frequency fields. Half of the trials were oriented so that north was to the left and the other half with north to the right. The results for male and female flies were analyzed separately given that male flies have been shown to show a significantly stronger magnetotactic behavior than the females. The average vial exit point for males (N/R = 4.9 ± 0.2 ; N/L = 4.9 ± 0.2) and females (N/R = 5.2 ± 0.2 ; N/L = 4.7 ± 0.2) were not significantly different from each other ($p > 0.1$) or from the expected value ($p > 0.9$). While this study reveals no innate directional preference in *Drosophila*, or a significant magnetic orientation behavioral difference between male and female flies, to the Earth's ambient magnetic field, it opens up many avenues for future research of magnetic orientation behavior in *Drosophila*.

Introduction

Drosophila melanogaster, more commonly known as the fruit fly, has been a model organism for behavioral and genetic research since first popularized by the research of scientist Thomas Hunt Morgan in the early 1900s. Currently, *Drosophila* are used in many branches of research in the field of biology, including magnetoreception. Magnetoreception is the ability of an organism to sense and use the information given by magnetic fields for orientation purposes. There are three aspects of the magnetic field that can be utilized by animals: the inclination of the magnetic field relative to the surface of the Earth, the direction to the magnetic north, and the local intensity of the magnetic field (Frings, 2008).

Organisms can “see” or “feel” the geomagnetic field and use that information to orient themselves during long distance travel. For navigation purposes, there are two main questions which are (1) Where am I? and (2) Which direction leads to my destination? Instead of receiving this information from a single sensory system, animals use a variety of sensory strategies to get information about their location and which way they need to go to reach their ultimate destination. There are two main hypotheses that attempt to explain the phenomenon of magnetoreception and the biological mechanisms behind it. The first is the magnetite mechanism and the second is the radical-pair mechanism.

The magnetite mechanism, is based upon the microbiology and physical properties of magnetotactic bacteria (Frings 2009; Huizar et al., 2016). These bacteria were found to have strings of particles of magnetite (Fe_3O_4) that are able to form a

stable, single-domain magnetic dipole, which means that the strings aligned with the geomagnetic field, like a compass needle. It is thought that the strings or chains of magnetite are connected to the gates of ion channels embedded in the cell membrane. When these gates are triggered by the magnetic field, the ion channels open causing a change in membrane potential. This is just the start of the sensory cascade that leads to the use of the magnetic field for orientation and navigation. The magnetite mechanism is believed to be used in migratory animals, for it supplies positional information from the geomagnetic field for long-distance travel. However, the magnetite particles in organisms with very strong magnetoreception have been found to be very small and not aligned in orderly chains like they were predicted to be. Thus many questions remain when it comes to the magnetite model of magnetoreception.

The second mechanism by which organisms are hypothesized to detect the magnetic fields is through light-mediated reactions. This mechanism is often described as the 'radical-pair model' in which a specific cryptochrome photopigment absorbs energy from light to cause the formation of a radical pair of molecules, each having one unpaired electron. The electrons can be affected by the magnetic field, allowing the organism to orient and navigate accordingly (Frings, 2008). These radical-pair reactions have been shown to be influenced by magnetic fields with a strength less than or equal to 50 μT , which is about the strength of the Earth's magnetic field. It has been argued in other studies that the radical-pair model works in response to stronger magnetic fields as well (Yoshii *et al.*, 2009). The photoreceptors required for this mechanism have been found in the retina of migratory birds, in *Drosophila melanogaster*, and several other

organisms (Bolte *et al.*,2016; Gegear *et al.*,2008).

When it comes to *Drosophila melanogaster*, one of the first studies exploring how they respond to magnetic fields was done by Phillips and Sayeed in 1993, which examined how *Drosophila* react to magnetic fields under different wavelengths of light. To explore the response of *Drosophila* to magnetic fields, they trained the flies for several days in a light gradient chamber (365 nm) in which no outside magnetic field was applied, so the flies would only respond to the Earth's magnetic field.

After training, the flies were put in a radial eight-armed maze lit from above by a lamp that either allowed 365 nm or 500 nm through as those wavelengths correspond to the most common absorption maxima found in the photoreceptors within the eye of *Drosophila*. The maze itself was centered atop a magnet and coil system (which produces a uniform magnetic field) that allowed the researchers to test the flies with the magnetic north to the north, south, east, or west. This entire setup was then grounded using a Faraday cage, which made sure that no outside magnetic fields would interfere with the flies' responses within the maze.

Their results showed that a statistically significant amount of flies that had been trained and tested under the 365 nm light wavelength exhibited magnetic orientation in the trained direction. However, those that were trained using 365 nm light and tested with the 500 nm light exhibited magnetic orientation that was shifted about 90° clockwise to their original trained direction. These results were only found within the male flies and not the female flies, which had no significant magnetic orientation in any of the trial conditions. Although this might be due to the difference in sex, another

alternative given is that the males and females responded differently to the light gradient used in the training regimen.

By training these flies under 365 nm light, this study was able to demonstrate that *Drosophila melanogaster* most likely use a light-dependent magnetic compass and that the rotation in direction was due directly to the change in the light wavelength. This rotation suggests that under the 500 nm light, the flies may be switching to another form of magnetic orientation behavior that has yet to be fully studied. From this study, the light mediated model of magnetoreception was further supported as the mechanism of magnetoreception in *Drosophila melanogaster*. However, the photoreceptor itself was not fully identified.

A study done by Gegeer *et al.* (2008) showed that the ultraviolet-A/blue light photoreceptor cryptochrome (CRY) is necessary for the light-dependent magnetic responses in *Drosophila melanogaster*. CRY functions in circadian rhythm regulation and detects light with a wavelength between 350 nm and 400 nm. The experiment used a binary choice T-maze to test the flies, in which the flies have to make a choice to go left or right. In this case electric coils were used to generate a magnetic field in one of the “arms” of the maze but not the other. *Drosophila* that had never been exposed to the maze before were tested as well as flies that had been trained in the maze with a sucrose reward when they went towards the arm with the magnetic field. The results from this experiment showed that *Drosophila* consistently went towards the magnetic field in both trained and untrained conditions. Yet, when different strains of *Drosophila* were tested and compared, it was found that various strains exhibited different

strengths of their magnetic preference. They also looked at these results to see if white-eyed or red-eyed flies performed differently in the maze, but they found no significant difference, indicating that eye color does not alter response to the magnetic field.

Next, they wanted to determine what would happen to the flies' behavioral response if light with a wavelength lower than 420 nm was blocked within the maze. They utilized the best performing strain and ran both trained and naïve flies through the maze. They found that when wavelengths lower than 420 nm were blocked, both the trained and naïve flies lacked their responses to the magnetic field that they had displayed previously. This data suggested that *Drosophila melanogaster* indeed do have a photoreceptor-based mechanism of magnetoreception and that CRY most likely functions as the magnetoreceptor.

The next part of the Gegear *et al.* (2008) study examined what would happen to the magnetic field response if fly mutants that lacked the CRY gene altogether were put through the maze. They used two types of mutants (*cry⁰¹* and *cry⁰²*), and they both lacked naïve and trained responses to the magnetic field during trials. They also ran flies through the maze that had a non-functional copy of the CRY gene and found that they were unable to respond to the magnetic field as well. These results further supported the idea that the CRY gene is an integral component of the magnetoreception pathway in *Drosophila*.

Gegear *et al.* (2010) explores the various types of cryptochromes that are found in the animal kingdom and how the different types are not all functionally the same. Type 1 CRY is sensitive to UV-A/blue light wavelengths and functions mostly as a

circadian photoreceptor; however, the type 2 CRY functions mostly as a negative regulator of the circadian clock's transcriptional feedback loop, which is a core component of the clock mechanism. Insects have been found to have only type 1, only type 2, or both type 1 and type 2. On the other hand, vertebrates only have type 2 CRY present. By examining the *Drosophila*-like type 1 CRY and the vertebrate-like type 2 CRY that is found in the monarch butterfly (*Danus plexippus*), they found that the CRYs mediate light-dependent magnetoreception through an unconventional photochemical mechanism.

Monarch butterflies have both type 1 and type 2 CRYs, and researchers wanted to see how each CRY type affected the behavioral response to the magnetic field. They inserted various monarch butterfly transgenes into *cry^b* fruit flies (in which FAD binding is impaired). It was found that the monarch type 1 CRY, like the *Drosophila* type 1 CRY, rescues the light-dependent magnetosensitivity mechanism in the flies. When the monarch type 2 transgene was tested, both the trained and naïve magneto-sensitive responses were restored as well in a light-dependent manner similar to the monarch type 1 transgene. Together these results support the idea that both monarch *cry1* and *cry2* proteins undergo the photochemical reactions that are necessary for the magneto-sensitive response seen in fruit flies.

Overall, there have been numerous studies done on the subject of magnetoreception in organisms that use either magnetite or the radical-pair theory to orient themselves. Even with all of this data available, there are still many questions about the intracellular mechanisms and processes that allow an organism to detect and

alter their behavior due to the presence of a magnetic field, whether that be the Earth's or an induced field in an experimental setting. The role of magnetoreception in *Drosophila melanogaster* behavior is not known, although there is a lot of data on the cellular mechanism that is used by them for magnetoreception. Another question involving *Drosophila* is whether or not they have an innate preference when it comes to magnetic fields. Are they naturally more likely to orient to the north or south, or do they have a preference at all? This has yet to be studied thoroughly by researchers and will be pursued in this study to gain a better understanding of the behavior of *Drosophila melanogaster* to the Earth's magnetic field.

This current study aimed to determine if *Drosophila melanogaster* have an innate directional preference utilizing a sequential Y-maze. The maze itself was housed within a Faraday cage, the purpose of which was to block out any radio frequency (RF) fields that may affect the choices of the flies. RF fields can be emitted from electronic equipment and there have been conflicting studies on whether these fields can affect the light-mediated magnetoreception found in *Drosophila* (Dommer *et al.*, 2008; Gegear *et al.*, 2008; Gegear *et al.*, 2010). Given the results of Phillips and Sayeed (1993), the choices for the male and female flies were analyzed separately and compared to evaluate for a difference in innate magnetic directional preference based on sex.

Methods and Materials

Flies:

The wild-type population of *Drosophila melanogaster* utilized in this study was obtained from a composting site in Monmouth, Oregon. The population was then kept and proliferated in the lab for the duration of the experiment (Generation 0). The flies were maintained in a 12h:12h light:dark cycle at 25°C on a standard dextrose medium supplemented with 0.1% Nipagen to inhibit mold growth. Selected populations of flies were also maintained under the same regimen.

Maze Design:

In order to determine if wild-type *Drosophila melanogaster* have an innate magnetic directional preference, a progressive Y-maze was constructed to allow the flies 10 directional choice points to either go North or South based on the ambient magnetic field in the experimental room (Figure 1).

The maze was constructed from plastic tubing with an inner diameter of 5 mm and an outer diameter of 8 mm. The tubing was connected with plastic Y-connectors and standard pipette tips were cut and inserted in each of the Y-connectors. The pipette tips were large enough to allow the flies to pass through them, but prevented them from going backwards in the maze, making sure that each fly only made 10 choices.

The entrance and the exit points of the maze were fitted with foam stoppers with holes into which the tubing was inserted. These stoppers were then inserted into

the collection and start vials. This allowed the flies that exited the maze to be held in the collection vials and sustained with food until they were examined and collected. The start vial that was initially connected to the maze did not contain any food, encouraging the flies to exit the vial and enter into the maze. On average, 100 flies were placed in each start vial prior to each run, although a small number of these would not survive or make it through the maze in time to be counted and thus were not included in the data.

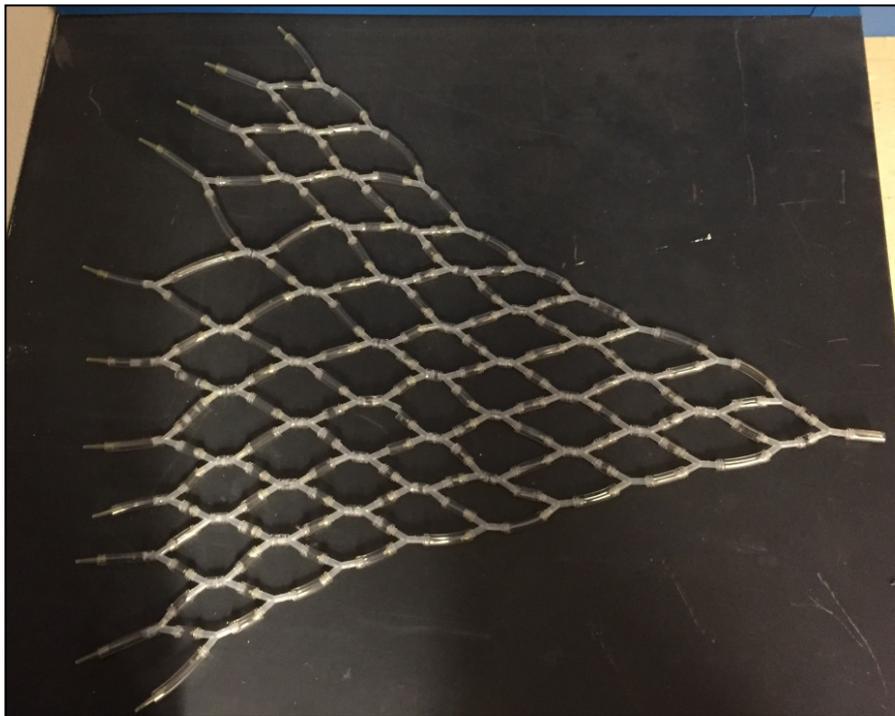


Figure 1: The maze used to run the flies during the duration of the experiment

Faraday Cage Design:

The maze was put inside a Faraday cage during the experimental runs to block out any interfering magnetic or electric fields within the building (Figure 2). This allowed the ambient magnetic field to be the only signal that the flies would respond to while in

the maze. The Faraday cage was constructed from a wood frame and aluminum wire mesh completely surrounding the frame. Aluminum has a conductivity of 36.9×10^6 Siemens/m. The wire mesh was wrapped around the frame to ensure that electric fields from surrounding equipment or the building itself did not interfere with the flies' choices.



Figure 2: The Y-maze contained within the Faraday cage

Experimental Set-Up:

Each run of the experiment was set up so that one side of the maze was north and the other was south. In some runs north was to the left and in others, north was to the right (Figure 3)-with direction randomly determined. If north was oriented to the right, then flies that oriented towards the north are making 10 choices to the right, whereas south-oriented flies would make 0 choices to go to the right.

The ambient light for each run was created using two 40 W desk lamps that were

directed upwards. Over the Faraday cage and maze, two fluorescent plastic light diffuser sheets were placed to ensure a uniform light gradient. If a bulb from one of the lamps was out when we went to collect the flies, that run was not counted or included in the final data set.

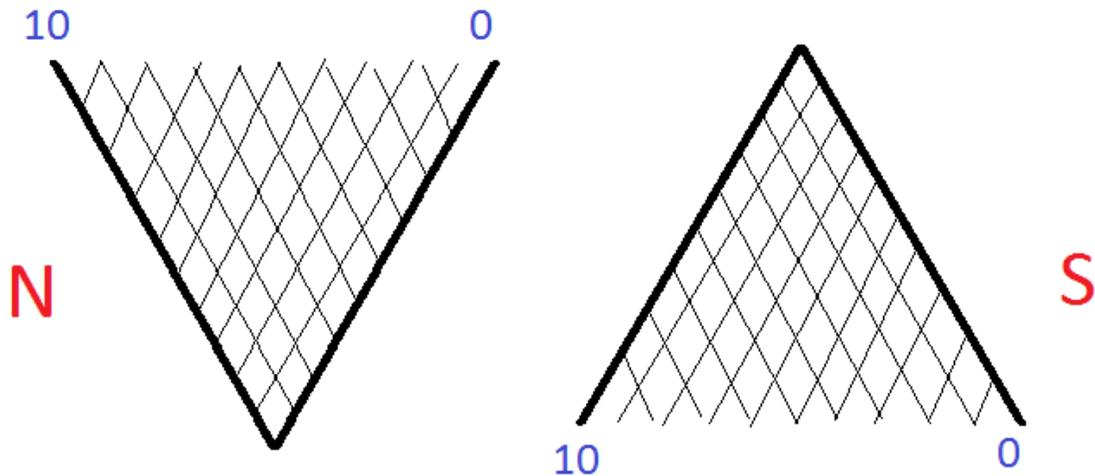


Figure 3: A diagram outlining the two orientations of the maze, north to the left and north to the right, utilized in the experiment with the corresponding values given to the vials.

Data Collection:

After an experimental run, flies from the vials numbered 0 through 10 were collected and then anesthetized with CO₂ to be counted using a dissecting microscope. The numbers of male and female flies was then recorded for each vial.

To determine whether the flies had an innate magnetic directional preference, 20 runs through the maze were performed. The data that was collected for each run was averaged to find the average vial number for each run. These averages were then analyzed using ANOVAs and Chi-squared tests in Microsoft Excel.

Results

The experimental runs and data were first categorized into groups including 1) males with north to the right, 2) females with north to the right, 3) males with north to the left, and 4) females with north to the left. The average vial number for the males with north to the right was 4.9 ± 0.2 and for females with north to the right was 5.2 ± 0.2 . For males with north to the left, the average was 4.9 ± 0.2 , and females with north to the left was 4.7 ± 0.2 (Figure 4). There was not a significant difference between groups ($p > 0.1$).

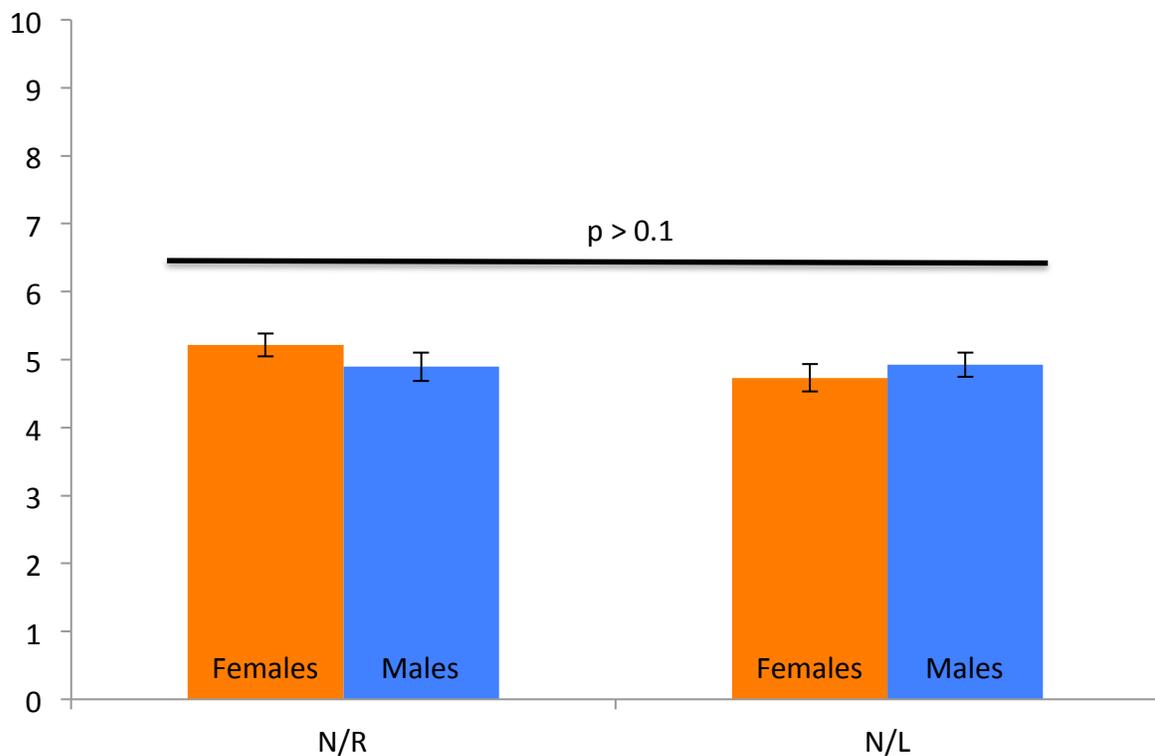


Figure 4: A comparison of the runs with north to the right ($n = 10$) and north to the left ($n = 10$). The error bars represent the SEM for each group.

Second, the flies were compared for a preference for left versus right within the maze. The data was standardized similarly to the north versus south comparisons with 0 being the most leftward vial in which the flies made zero right choices in the maze and 10 being the most rightward in which the flies made 10 right choices within the maze. The average vials for the males with right to the north (Group 1, 4.0 ± 0.2), females with right to the north (Group 2, 5.2 ± 0.2), males with right to the south (Group 3, 5.1 ± 0.2), and females with right to the south (Group 4, 5.3 ± 0.2). The averages for each of the individual runs were analyzed using an ANOVA, (p -value > 0.5 , Figure 5).

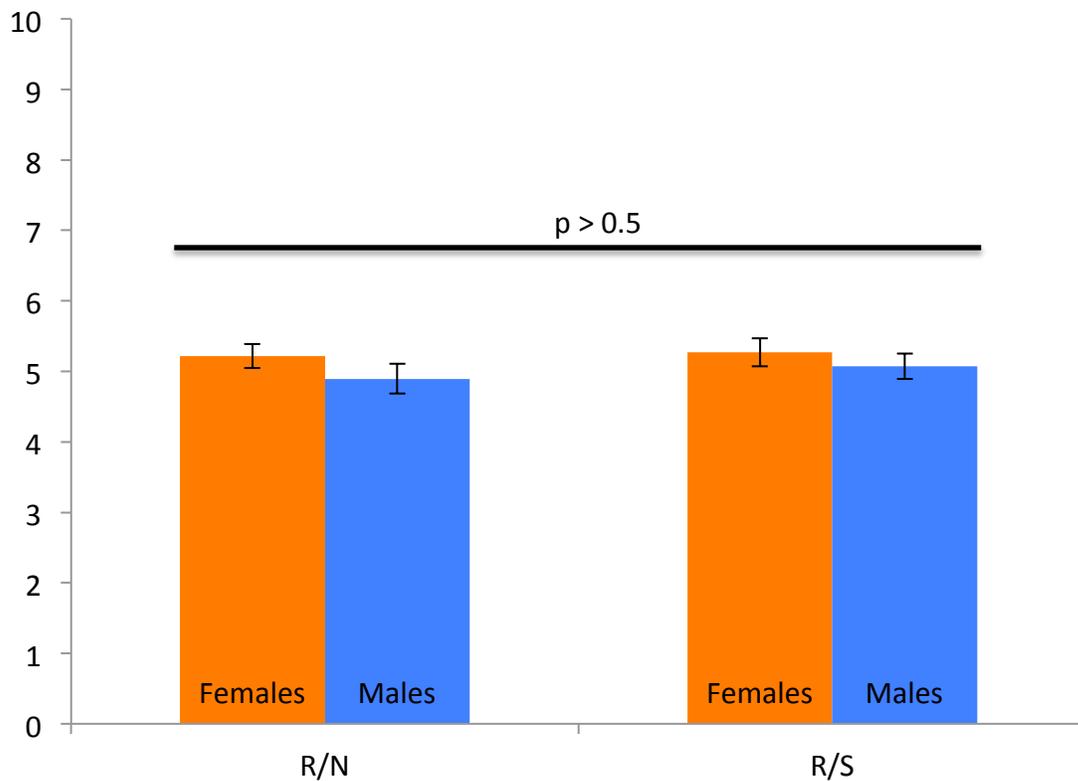


Figure 5: A comparison of the runs with right to the north ($n = 10$) and right to the south ($n = 10$). The error bars represent the SEM for each group.

Lastly, a Chi-squared test was performed with the averages of the pooled data of all of the flies with north to the right (average = 5.1) and north to the left (4.9) in comparison with the random distribution expected value of 5.0 (which would indicate that the flies have no significant directional preference). This analysis indicated that the data was not significantly different from a random distribution ($p = 0.9$).

Discussion

The analysis of the data after 20 runs of naïve, non-trained flies through the maze revealed no significant preference for north or south within the 10 choice point Y-maze (ANOVA, $p > 0.1$, Figure 4). There was also no significant difference in the magnetic directional preferences between males and females. Phillips and Sayeed (1993), showed that male *Drosophila melanogaster* exhibit a magnetic directional response after training in a chamber in which the magnetic field is aligned with a 365 nm light source. The data from this current experiment suggests that *Drosophila melanogaster* do not have an innate magnetic directional preference, which is consistent with previous studies that have required training before the flies have responded to a certain direction and/or magnetic field in general.

The analysis of left versus right choices in the maze was also not significant between sexes and maze orientation (ANOVA, $p > 0.5$, Figure 5). This result indicates that the flies do not have a right or left directional preference within the maze. The averages for the north/south groups and the right/left groups were not significantly different from the expected value of 5.0 when analyzed using a chi-squared test ($p > 0.9$). This data indicates that there was no bias within the maze itself from its construction or other properties that could possibly alter the flies' behavior and their choices while within the maze.

As mentioned in the introduction, many of the previous studies done with *Drosophila melanogaster*, including Dommer et al. (2008), Gegear et al. (2008), and Phillips and Sayeed (1993) all utilized applied magnetic fields in their experiments, and the flies were trained to prior to being tested. The applied magnetic fields used were sometimes much stronger than the ambient magnetic field of the Earth, which is about 50 μT . For example, the field used in Yoshii et al. (2009) was 300 μT , which is 6 times stronger than the average Earth's magnetic field. This, along with the present study indicates that although *Drosophila* may be trained to respond to a

strong applied magnetic field, they may not be able to detect and/or respond to Earth-strength magnetic fields.

Previous research with *Drosophila melanogaster* has shown that various traits, such as positive and negative phototaxis can be artificially selected for using a similar Y-maze with 15 choice points (Hadler, 1964). We are currently performing an experiment in which north and south selected flies are bred through 15 generations to determine if there are genetic underpinnings to the magnetoreception in *Drosophila*. We have begun this experiment, and while no significant preference for north or south with wild-type flies has been shown (James *et al.*, 2016), it could possibly be selected for over an increased number of generations. Also, another endeavor in the future could use a similar experimental setup as this study except using a stronger applied magnetic field to see if the flies have an innate preference to magnetic fields that are several times stronger than Earth's ambient field.

Even though the results of the current study show no significant innate magnetic directional preference in wild-type *Drosophila melanogaster*, there are many areas of research open to investigate in the future. As was shown in the Gegear *et al.* (2008) study, *Drosophila* mutants for certain *cry* genes (*cry*⁰¹ and *cry*⁰²) did not have either a trained or naive magnetic response, so designing an experiment running them within a maze like the one in this experiment and comparing it to the wild-type flies could give us a baseline comparison between those flies that have the ability to detect and respond to magnetic fields and those that cannot. Also, using other mutants that have been known to show an altered magnetic response to run through a maze similar to our set-up would give us more information as well. Another experiment worth pursuing would be to see if flies collected from different geographical locations and/or different strains of *Drosophila*

naturally respond differently to the Earth's ambient magnetic field. Flies in different areas, or different strains, may have had selective pressures to have a north or south preference over time, which could result in a preference that was not seen with the flies used in this experiment.

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