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Selection of a 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

Diverse organisms, including birds, sea turtles, lobsters, and sharks have been shown to use Earth-strength magnetic fields to navigate. We have examined whether the fruit fly *Drosophila melanogaster* has a directional preference and if this preference has genetic underpinnings. In order to answer these questions we designed a Y-maze in which each fly makes 10 sequential choices to go North or South. To breed a population of North-seeking flies, we recorded the distribution of flies exiting the maze and selected the Northern-most 20% to give rise to the next generation. We used a similar protocol to breed a population of South-seeking flies. Our data showed that wild-type *Drosophila* do not have a distinct innate preference for either North or South. Moreover, after 12 generations we did not produce a population of flies with a distinct directional preference. As a positive control we ran a similar experiment to look at phototaxis. Our data showed that flies exhibit positive phototaxis and after 12 generations of selective breeding we have produced a strain with a decreased phototactic response. These experiments will be continued for 15 generations. Our findings will contribute to a better understanding of the magnetic orientation behavior of *Drosophila*.

Introduction

In behavioral biology, one class of phenomena that is frequently observed is that of taxis. Taxis is the directional movement or orientation shown by an organism in response to a stimulus. This stimulus can come in many forms including: light, sound, chemicals, wind, and gravity¹. One type of taxis that is dependent on a magnetic field, magnetotaxis, is related to a concept known as magnetoreception. Magnetoreception is the ability for an animal to detect and respond to magnetic fields and has been demonstrated by many diverse organisms. Migratory birds that use the Earth's magnetic field to navigate as they travel long distances are the most commonly known example of an animal that uses magnetoreception². Similarly, loggerhead sea turtles are also known to use magnetic fields as they migrate across the ocean^{2,3}. Arthropods such as the spiny lobster have also demonstrated that they can use magnetoreception to navigate, and can use it on a very localized scale, being able to find their way back to their den when other navigational cues are very limited^{2,3}.

Though magnetoreception has been observed in all of these diverse animals, there is still much to be understood about the fundamental mechanisms that allow for such an ability to exist^{2,3,4,5}. For decades it was a mystery as to how the magnetic fields were being perceived and what the receptor was that could detect them. Because of this, magnetoreception has been the subject of many

behavioral experiments and studies with the intentions of finding out more about the mechanism by which it acts, as well as identifying which species possess the capability⁴. One reason that the neural basis for magnetoreception has been elusive is that species that are known to exemplify magnetoreception robustly are unpractical subjects for laboratory experiments examining behavior. Instead of attempting to use an organism like a sea turtle in the lab, many studies have consisted of running tests on already known model organisms to determine if they have the capability of magnetoreception. For example Phillips *et al.* (2013) used laboratory mice in a water maze experiment where a magnetic field was present⁶. Mice were trained by giving them a chance to learn how the magnetic field was associated with the safe zone of the maze. Their results supported that mice can use magnetic fields to carry out localized tasks, which is important because mice are much easier to work with than large migratory animals, such as sea turtles.

Another well-known and widely used model organism is the fruit fly *Drosophila melanogaster*, which is commonly celebrated for its short generation time, limited nutritional requirements, and ease of genetic analysis and manipulation. Because fruit flies have been used for over a century in biological experiments, there is a vast library of information detailing their behaviors, physiology, and genetics and they therefore often serve as ideal subjects for

many different types of research studies. So it is no surprise that there have been several studies that have looked at fruit flies to see if they have the ability to detect magnetic fields. In 1993, Phillips and Sayeed conducted a study that trained flies to associate a light source with a compass direction⁷. They found that male flies, but not female flies, did show a response and preferred to go toward the direction that was associated with the light source during training.

In the past 20 years, there have been many developments and insights gained into the fundamentals of magnetoreception, including the receptor responsible. Today there are two prominent mechanisms proposed to be responsible for magnetoreception, one of which is thought to be more widespread than the other. The magnetite model involves a receptor that is associated with permanently magnetized particles of Fe_3O_4 , which would serve as the material by which the magnetic field could act upon⁴. Alternatively, the radical-pair model, which is proposed to be the more widely used mechanism across the animal kingdom, is light-dependent and involves the excitation of electrons, creating free radicals that can be modulated by a magnetic field, allowing for the field to be detected by a receptor. The magnetite model is thought to be more useful for gaining positional information, while the radical-pair model is better suited for determining compass directions⁴. The protein that is the top candidate for the radical-pair mechanism is the blue light receptor,

Cryptochrome (CRY). CRY was first discovered in the plant *Arabidopsis thaliana*, but has since been identified in all domains of life and is widespread across the animal kingdom. In animals it was primarily known to be associated with regulation of the circadian rhythm, and is thought to be responsible for magnetic field detection as well⁸. Yoshii *et al.* (2009) found that variations in magnetic field strength as well as wavelength of light would affect fruit flies circadian rhythms, while mutant flies that lacked CRY did not show a significant response. This showed support for both the claim that CRY is sensitive to magnetic fields and that magnetic fields can affect the behavior in fruit flies⁹.

Because CRY is so prevalent in animals, it is reasonable to hypothesize that there could be many species that use magnetoreception, potentially for more localized behaviors, rather than large-scale migrations². This has been shown in nudibranch sea slugs, which are thought to use magnetoreception to navigate the intertidal zones and effectively find food^{2,3}. Alerstam (2006) argues that because magnetoreception can be obviously present in one species, while apparently absent in another closely related species, magnetoreception must be an ability that can, on an evolutionary timescale, be quickly activated or repressed through natural selection².

The study presented in this paper focused on selecting for a magnetoreceptive response in fruit flies by breeding subsequent generations

from flies that went either towards the North or the South in a Y-maze. There have been other studies that have examined the innate ability for flies to orient based on magnetic fields^{7,10,11}, but they have given some conflicting results, e.g. one study showed that only male flies show a response⁷ and another that only specific genetic strains show a response¹¹. The goal of this study was to take wild-type flies and strengthen any magnetic orientation capabilities that they may possess through selective breeding. A similar procedure was used in a fruit fly experiment that examined phototaxis, which used a Y-maze and a variant light gradient to separate flies based on how they responded to the light¹². Those that went most toward the light were used to breed a population of light-selected flies, while the same thing was done to breed dark-selected flies. The study found that after 10 generations, light-selected flies showed a stronger preference for the light side of the maze, whereas the dark-selected flies preferred the dark side when compared to wild-type flies¹². If using a similar procedure we produced populations of flies that preferred North or South, this would support the hypothesis that fruit flies have some behavior that is dictated by magnetoreception. A population of flies that was bred to prefer a particular compass direction would also be a valuable model organism for future experiments that could look at the genetic and molecular factors that are associated with magnetoreception.

Methods

Maze Construction:

We built a Y-maze based on the design of a maze that was used to test flies for phototaxis in a previous study¹². We used clear aquarium tubing to join plastic Y shaped connecting joints. To make the maze unidirectional and prevent the flies from backtracking through the maze we inserted modified 200 μ L micropipette tips into each choice point of the maze. The pipette tips were cut to be large enough for a single fly to pass through at the end. The completed maze had 11 different possible endpoints where 25 x 95 mm collection vials were connected to the maze using punctured foam stoppers. To encourage flies to reach the end and prevent them from desiccation, each end vial contained a small amount of fly medium. Flies were loaded into the maze by connecting a single vial to the entrance.

Set-up procedure:

Approximately 50-200 flies were transferred to a clear, empty starter vial without using an anesthetic. For North vs. South trials, the maze was oriented so that the extreme right and left sides of maze exit points corresponded to North and South. The orientation of the maze was pseudo-randomized so that for some trials North was to the right and for other trials North was to the left. This was

done to avoid selecting for flies that simply went right or left rather than North or South. The start vial was attached to the entrance of the maze and flies were given approximately 24 hours to run through the maze. Because *D. melanogaster* has been shown to be phototactic, the lighting of the room and maze was controlled by suspending light diffusing sheets approximately 9 inches above the maze and the only light sources in the room were a pair of 40W incandescent lamps. For our phototactic positive control trials, we set up the maze to have a light source on one side, left or right, by placing a 40W lamp behind a vertically oriented diffusor.

Fly/Data Collection and Breeding:

24 hours after entering the maze the flies were collected, and then the maze was examined to remove any flies that were still inside to prevent any blockages for future maze runs. The flies were counted by anesthetizing them with CO₂ gas and the number of flies in each vial was recorded as raw data. While counting the flies, the counter was 'blinded' as to which population they were counting. As part of our breeding procedure, the strain of flies (North-, South-, Light-, or Dark-selected) was revealed to the counter after data was collected, and the top 20% of flies that went most toward the selected direction were transferred to a new vial with fly medium and activated yeast to become the parents of the next

generation of flies for that strain. Flies were maintained in a 25°C incubator with 12hr:12hr light-dark cycles on standard dextrose medium with 0.1% nipagen to inhibit mold. Every 2 to 3 weeks the maze needed to be washed with hot water so that any aromatic or chemical trails left behind by the flies were removed.

Statistical Analysis:

The number of flies in each vial was counted and recorded after each run and the mean value for that run was calculated using the weighted average of the distribution. ANOVA and two tailed t-tests were done to determine if the difference between two runs was significant beyond random chance and a significance standard of $p < 0.05$ was used.

Results

After breeding flies for 12 generations and selecting for directional preferences toward North, South, Light, and Dark, the following data describing their behavior through the maze was obtained. After running each strain through the maze, the flies were collected and the number in each of the 11 vials was counted.

The distribution of North and South strains varied from our starting wild-type generation 0 through to the selectively bred generation 12 (Figure 1). When

examining the distribution for patterns, there is not a consistent trend of progression shown in the flies' distribution from generation to generation. Rather, the values seem to fluctuate up and down, with most of the values falling between 3 and 7. There are 5 generations in which the North selected strain's distribution was more North than the South, there are 4 generations in which the South selected strain's distribution was more North than the North, and there are 3 generations in which both strains showed approximately the same mean distribution.

After 12 generations of the Light and Dark selection experiments the range of mean values for most of the generations of flies fell between 5 and 9, with one run having a mean distribution of 4.4 (Figure 2). The distributions show more choices, on average, toward the side of the maze where the light source was positioned (Figure 2). There are 6 generations in which the Light selected flies had more distribution toward the light source than the Dark selected flies and there are 6 generations in which both strains showed approximately the same distribution toward the light.

The ANOVA results show that there was a significant difference between the runs of generation 12 compared to generation 0, and this analysis included comparisons between the North/South trials and the Light/Dark trials, indicating

that, statistically, there are differences in the behavior of the flies under the two experimental conditions as well as across the generational gap.

Generation 0 wild-type control runs were done using multiple maze orientations in order to obtain a mean value of random distribution for the maze that was independent of external factors that could influence the flies' behavior. Because of this there are North/South (N/S), South/North (S/N), and East/West (E/W) data sets for generation zero. Two-tailed t-tests showed a significant difference between both generation 12 North and South populations when compared to the generation 0 S/N trial (Table 2). However, North and South generation 12 results did not have p-values less than 0.05 when compared to the other two generation 0 runs (Table 2).

Two-tailed t-tests also showed that there was a significant difference between the generation 12 Dark selected flies when compared to the generation 0 L/D trial, indicating a difference in distribution and a potential change in behavior over the 12 generations of selection (Table 2). There was not a significant difference between the generation 12 Light selected flies and the generation 0 L/D trial (Table 2).

Discussion

The results given by the ANOVA of all Gen 0 and Gen 12 results does not give any specific information about a progressional change in behavior, but what it does show is that there are significant differences between the two data sets, meaning that something more than random chance is causing the flies to behave differently (Table 3). The inclusion of the Light/Dark data in the ANOVA is likely to be the reason for some of the significance, due to the effect that an uneven distribution of light had on the flies' behavior, but the significance could also come from a change in the distributions from generation 0 to generation 12.

The behavior of the flies and the distribution that they displayed throughout the experiment was not as consistent as we predicted it to be in the early planning stages of this experiment. The mean value of distribution from generation to generation fluctuated for both the North/South trials and the Light/Dark trials, meaning that either the flies have less predictable behavior than expected, or there were inconsistencies in the conditions of the maze from one run to another (Figure 1, Figure 2).

For the North/South trials, there are only 5 out of 12 trials in which the North selected flies actually went more North than the South selected flies (Figure 1). By superficially looking at the Figure 1 results, it appears that we were not successful in selectively breeding strains of flies that showed a directional

preference for either North or South. However, these figures show only the *mean* of the distribution, and not the variance. The t-test results take into account the variance of the distribution of each run and did show that there was statistical significance to the generation 12 North and South strains when compared to one of the initial generation 0 trials (the Gen 0 S/N) (Table 2). But comparing the generation 12 North and South strains to the other two generation 0 trials does not yield the same significance (although the generation 12 South strain came close when compared to the E/W trial). Moreover, the generation 12 South-selected strain showed a mean distribution that was more North than the North-selected strain. Because of these inconsistencies it is not possible to definitively claim that we selected for a directional preference within these 12 generations, and the flies either don't respond to a selective pressure based on North and South or they require more than 12 generations before they begin to show a separation of preference.

The distribution data for the Light/Dark trials also fluctuated, but the mean values indicated that the flies showed positive taxis toward the light, which was expected (Figure 2). The manner in which the mean distribution varied from generation to generation makes it seem that the selective breeding was working for the Dark-selected strain during certain generations, but for other generations both the Light and the Dark strains had similar mean distributions (Figure 2). But

as it was discussed above, these figures only depict the mean, not the variance. According to a t-test, the generation 12 Dark-selected strain showed a variance that was significantly different from the generation 0 L/D control run, while the Light-selected strain did not. This could indicate that the selective breeding worked to create a strain of flies that had a weaker phototaxic response than the wild-type. The fact that only the Dark-selected strain, and not the Light-selected, showed a significant difference could indicate that the phototaxic response is more easily weakened than it is strengthened, since the behavior is already fairly robust in the wild-type flies. Successfully breeding a Dark-selected strain was also important to the validity of the experiment as a whole, since phototaxic selection was the positive control we used as a test for our methods. Because of this success we can conclude that our maze design can work for directional selection experiments.

Though each run included approximately 50-200 flies, the mean distribution of each run is treated as a single data point. This is because the flies do not behave independently while in the maze and some evidence of following behavior was observed. This was most clearly seen when counting the number in each vial to find a completely empty vial between two other vials that each contained a significant number of flies. For example, random chance would predict that vials 5, 6, and 7 would all contain at least some flies, but in some

cases vial 6 would have no flies while vials 5 and 7 would each have a significant number. Additionally, because we only ran each generation of flies through the maze once, our results are based on pseudo-replication. To account for this, when this experiment reaches generation 15, 10 replication runs will be done for all generation 15 strains and 10 additional control runs will be done using generation 0 wild-type flies.

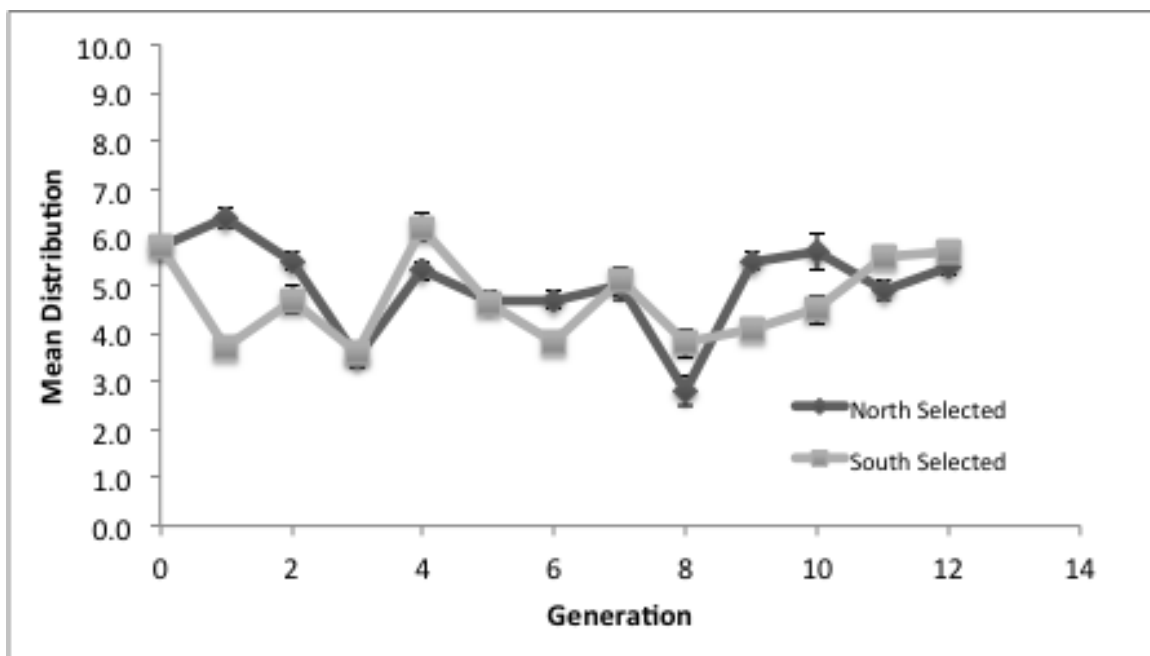


Figure 1. Comparison of the mean distribution of North and South populations for generations 1 through 12. Distribution value indicates number of North choices through the maze. Error bars indicate SEM.

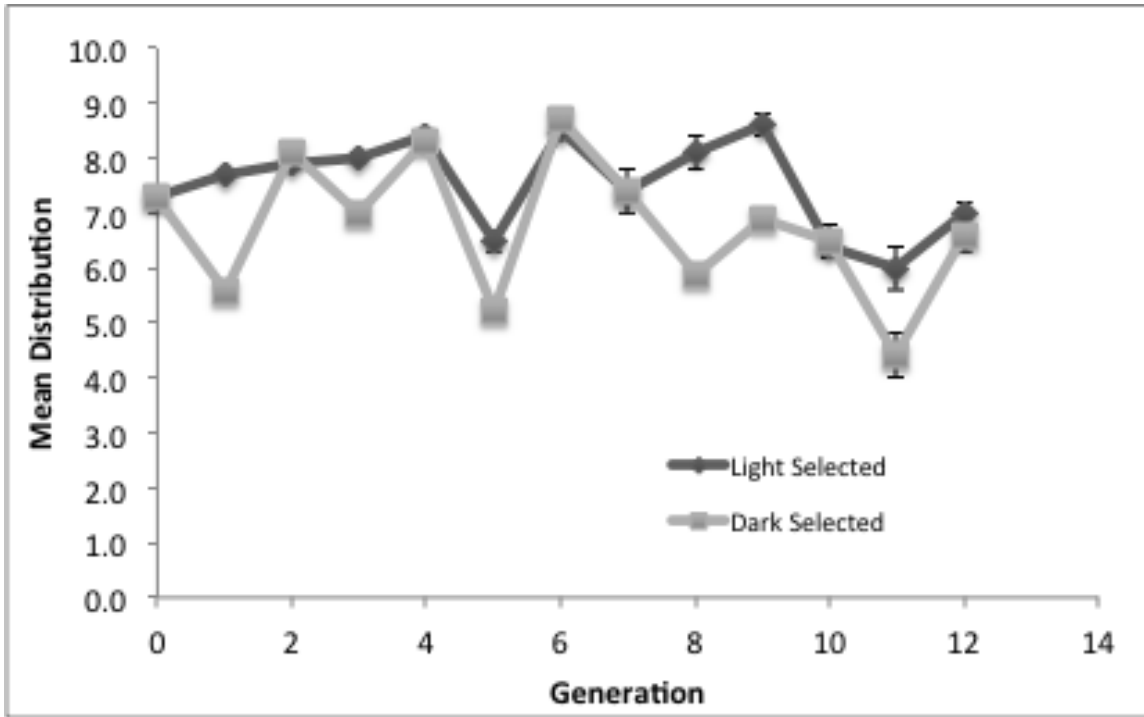


Figure 2. Comparison of the mean distribution of Light and Dark populations for generations 1 through 12. Distribution value indicates number of Light choices through the maze. Error bars indicate SEM.

Table 1. Results of two-tailed t-tests assuming unequal variance comparing all strains from generation 0 to all strains of generation 12. $p < 0.05$ is significant.

t-tests	Mean	L/D Gen 0	Light Gen 12	Dark Gen 12	S Gen 12	N/S Gen 0	N Gen 12	E/W Gen 0
S/N Gen 0	4.0	1.79×10^{-21}	3.29×10^{-17}	5.19×10^{-12}	5.59×10^{-8}	5.61×10^{-4}	6.67×10^{-6}	0.016
E/W Gen 0	5.0	1.89×10^{-8}	1.11×10^{-6}	1.76×10^{-4}	0.057	0.280	0.233	
N Gen 12	5.4	7.57×10^{-10}	5.77×10^{-7}	7.57×10^{-4}	1.76×10^{-5}	0.902		
N/S Gen 0	5.5	1.34×10^{-5}	3.40×10^{-4}	0.013	0.582			
S Gen 12	5.7	3.66×10^{-8}	1.76×10^{-5}	8.39×10^{-3}				
Dark Gen 12	6.6	0.019	0.197					
Light Gen 12	7.0	0.239						
L/D Gen 0	7.3							

Table 2. Results of two-tailed t-tests arranged to compare Generation 0 control tests with the runs after 12 generations of selective breeding. $p < 0.05$ is significant.

Comparison of Selected Strains to Gen 0				
Generation 0		Generation 12	p value	p < 0.05
N/S	vs.	North	0.902	No
N/S	vs.	South	0.582	No
S/N	vs.	North	6.67×10^{-6}	Yes
S/N	vs.	South	5.59×10^{-8}	Yes
E/W	vs.	North	0.233	No
E/W	vs.	South	0.057	No (close)
L/D	vs.	Light	0.239	No
L/D	vs.	Dark	0.019	Yes

Table 3. Single factor ANOVA comparing all of Generation 0 runs to all Generation 12 runs.

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	605.53	7	86.504	19.077	4.07×10^{-23}	2.024
Within Groups	2784.14	614	4.534			
Total	3389.67	621				

Conclusions

After 12 generations of selectively breeding *Drosophila melanogaster* for both North & South preferences and Light & Dark preferences, and using statistical analysis of both the mean values and the variance of each maze run, we have found that we were unable to produce a fly strain that demonstrated a

directional preference for either North or South, but we were able to produce a strain of flies with a weakened phototactic response. This shows that we have developed an experiment that is able to select for negative phototaxis, and that this species does not show a magnetoreceptive directional preference, based on Earth-strength magnetic fields, that can be selected for in the same way a phototactic response can. From this we can conclude one of the following: 1) the flies do not possess any directional orientation behavior based on magnetic fields; 2) the experiment was not carried out for enough generations; or 3) that any orientation behavior they display is too subtle to be detected or acted upon by our experiment. This experiment is to be continued until generation 15 has been reached, as was part of the original experimental design. In future studies, this experiment could be modified to include an artificial magnetic field that is much stronger than the background field of the Earth, as has been done in many other magnetoreception studies^{6,7,9,10}.

Acknowledgments

I would like to share equal credit with fellow classmates Taylor James and Spicie Davis for their efforts and collaboration during the initial design stages of this experiment, for both our successful and less-than-successful maze designs, and the methods for how to get 200 fruit flies to run through a maze. I would also

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Supplemental Literature Review (for background information)

Magnetoreception is the ability for an organism to detect magnetic fields. The implications of this sensory ability have been an area of great interest amongst many different researchers. Studies have been conducted that focus on identifying both the responses that various organisms display when exposed to magnetic fields during specific tasks, as well as elucidating the mechanisms behind how organisms actually detect the magnetic fields. The types of magnetoreceptive responses that are commonly studied include: long distance migration, determination of a geographic location, orientation, and “training” organisms to use magnetic cues learned in a lab to solve a particular task. The mechanism by which the magnetic fields are detected has long been a mystery to many. However, recently there have been two main mechanisms presented: the magnetite based mechanism, and the radical pair mechanism. The fundamental concept of the magnetite mechanism involves microscopic, permanently

magnetic particles of magnetite (Fe_3O_4) that are associated with specific sensory neurons. The radical pair mechanism on the other hand, is a light-dependent mechanism that involves photoreceptors that produce unpaired electrons that can then be modulated by magnetic fields, making the magnetic field a detectable stimulus. Many studies involve examining both the types of responses that specific organisms display, as well as how those responses support either the magnetite model or the radical pair model of magnetoreception^{2,3,4,5}.

In a study by Phillips and Sayeed (1993), they examined the way that the fruit fly, *D. melanogaster*, behaved in the presence of magnetic fields⁷. Their experiment took place when the mechanism for magnetoreception was still unknown and the ability for fruit flies to detect magnetic fields was still unsure. The goals of their experiment were to see if the flies would show a specific behavior in response to a magnetic field after being 'trained,' and they also wanted to test the effects of varying light intensities to see how they might affect the outcome of the experiment. Their experiment was partially based off of a previous study by Phillips (1986) that had been conducted using newts¹³, which showed not only that newts will respond to a magnetic field, but also that depending on the wavelength of the light used they would show different responses. In Phillips and Sayeed's study flies were trained in a chamber that had 365nm light coming from one direction that was associated with a compass

direction: north, south, east, or west. Then they released the flies into a chamber with eight different pathways and a magnetic field that the experimenters could manipulate. Their data reported that the flies responded to the magnetic field by heading in the direction that was associated with the light source during their training; however, they found that only the males showed a significant response and the females were randomly distributed. They then repeated the experiment using 500 nm light during training and observed that the response to the magnetic field was shifted 90° clockwise. From these experiments they concluded not only that the flies do show some distinct detection and reactions to magnetic fields, but also that the response changes based on the wavelength of the light used in training. These results supported the early hypothesis that the mechanism of magnetoreception in flies was light dependent, which today matches up with the radical pair model. However, they also believed that the change in behavior could also be caused simply by the different light, triggering a behavior that would lead to a different reaction to the magnetic field, rather than the light changing how the magnetic field was perceived.

This study was valuable for multiple reasons. Not only did the authors develop an experiment that showed a clear magnetoreceptive response from *D. melanogaster*, but they also showed that the mechanism might be light dependent, setting up many opportunities for future studies. However,

something odd in their data was their report that only male flies exhibit the behavior, whereas other studies later on showed that both males and female flies show signs of magnetoreception.

In another study, Yoshii *et al.* (2009) looked at the effect that magnetic fields can have on the natural circadian rhythm (sleep cycle) of fruit flies⁹. As the study by Phillips and Sayeed (1993) showed⁷, the magnetoreceptive mechanism in the flies is light dependent. In other studies^{4,8,11}, the photoreceptor cryptochrome (CRY) was shown to be likely candidate for the radical pair mechanism. Cryptochrome is a blue light photoreceptor that, when excited by a photon, creates a radical set of unpaired electrons. The theory behind the radical pair mechanism is that a magnetic field can modulate the spin states of these unpaired electrons, allowing for the magnetic field to be detected by the animals visual systems. CRY has also been found to be associated with the circadian cycle of the fruit fly and other organisms⁸, so Yoshii *et al.* used the lengthening of the circadian rhythm as a measurable response to different magnetic fields. Their trials consisted of exposing the flies to different light and magnetic field conditions for several days, and then observing how the respective conditions affected their circadian cycles. Their study was thorough in that they ran tests using a variety of conditions to test multiple variables. Initial tests involved exposing flies to blue light (the wavelengths that are detected by CRY) and

magnetic fields of varying strength. From these tests they found that increasing the magnetic field had more of an impact on the circadian rhythms of the flies. They also ran trials that used red light instead of blue light. The flies exposed to red light in a magnetic field did not show a response, supporting the hypothesis that photoreceptor mediated magnetoreception in flies is dependent on certain wavelengths of light. This would be because the CRY wasn't activated and there were no radical pairs for the magnetic field to act upon. To further investigate the role that CRY plays in magnetoreception, they ran more blue light trials using mutant strains of flies. They tested a strain in which CRY receptors were absent, a strain with a mutant version of CRY, as well as a strain that would overexpress CRY. They found that the strains with mutant or absent CRY had no significant response to the magnetic field, whereas the strain that was overexpressing CRY had an increased response.

This was an important study because it provided insights into the role that CRY plays in both the circadian rhythm of flies and the detection of magnetic fields. By using the different conditions in the trials they showed that the magnetic field response was dependent on both the presence and activation of CRY, providing great support for the radical pair mechanism in *D. melanogaster*. In their paper they discuss that CRY is found in a wide diversity of organisms, including species of birds and even plants. They also bring up the alternative

interpretation of their results that the magnetic field is simply affecting the CRY and causing the flies to perceive certain wavelengths of light with a greater intensity than it actually is, rather than the stimulus being processed as something separate from sight. They also note that their study doesn't show anything to demonstrate the use of magnetic fields in any sort of orientation behavior, although the fact that CRY has been found in other body systems of fruit flies, as well as so many other animals, it is highly plausible that CRY could play a role in orientation.

The fact that CRY is so prevalent in the animal kingdom helps to answer a question that was presented by Alerstam (2006) about long distance migration in animals². In his paper he evaluates the observation that the ability to navigate using magnetic fields must be a very flexible adaptation, for a number of reasons. For example, the fact that some species of birds migrate hundreds or thousands of miles using magnetic maps, while other very closely related species don't migrate at all is perplexing. It seems as though magnetic migration is a trait that can easily be promoted or suppressed over evolutionary time. This is important because it has led to the speculation that large scale magnetic migration is based on a mechanism that is already present in most animals, which provides support for the argument that a vast number of non-migratory animals utilize magnetoreception for more localized behaviors.

Another observation that is perplexing is that the magnetic markers that are presumably used in migration are constantly changing. There are many animals, mostly birds, that have been known to migrate nearly half way around the world from the northern hemisphere to the southern hemisphere, but the magnetic poles of the earth are not forever constant, and over the course of just 50 years the magnetic field at various locations may change significantly, meaning that future travelers would be thrown way off course if they were using inherited directions from past generations. In order to keep up with the changing magnetic cues used in migration, it is hypothesized that the mechanism is rather simple and easily adapted in short evolutionary time scales. A likely possibility is that migrating animals utilize a number of different strategies in order to determine their course. Species of birds have already been known to use visual landmarks and celestial cues to find their way to their destination, and it's possible that they could use these methods of navigation as a means to recalibrate their magnetic map. Experiments have been done on migratory seabirds and sea turtles where they actually attached magnets to them in an effort to disrupt their ability to navigate with the magnetic field. However, despite the supposed interference, the birds and sea turtles were still able to find their way². Their success could be explained if they had the ability to recalibrate their navigation to compensate, which supports the idea that more than one navigation strategy is in play.

This article brings up two important points in regards to magnetoreception. For one, magnetoreception is likely an ability that is present in many animals and not exclusive to just migratory species. And second, the means by which magnetic migration is developed is likely a flexible mechanism capable of being modified both through evolutionary time across species, as well as from generation to generation of the same species.

The ability to use magnetic mapping for long distance travel has long been well documented in species of bird, however, in a paper by Cain *et al.* (2005) they highlight the importance of magnetic migration in underwater marine environments³. In the ocean, visual cues are not a practical means of navigation due to the drastically reduced visibility underwater and the fact that landmarks are few and far between. However, the earth's magnetic field is present everywhere, all the time, and is not greatly affected by conditions such as the weather, making magnetic migration highly advantageous for marine animals. There are two major components of magnetoreception that are important for navigation, one being the ability to determine and maintain a constant direction/heading, and the other being the ability to use the magnetic field to determine a geographic location. Loggerhead sea turtles have long been a classic example of a migratory marine species that use magnetic cues to navigate. Juvenile loggerheads have been seen to migrate thousands of miles across the

Atlantic Ocean. Careful navigation is important during their journey because straying off course would likely mean getting swept off course by a current and ending up in the colder water, which would be fatal. Because of this, a good technique for navigation provides a strong selective advantage for those that make the journey safely. They believe that not only can the turtles utilize the magnetic field to maintain a heading, but that they can also derive their position from the field as well. Because the earth's magnetic field behaves differently depending on latitude, the turtles can potentially use this to determine where they are when no other information is available to determine their position.

This type of true navigation has long been documented in vertebrate species; however, more recently it has been demonstrated in invertebrates, specifically the spiny lobster. The lobsters' daily foraging behavior typically involves them venturing out at night and returning to a den for the day. Experiments to test their ability to navigate involved capturing individual lobsters from their dens and transporting them several kilometers away to an area that was unfamiliar to them, and then observing whether they could find their way home. They found that these lobsters are indeed capable of true magnetic navigation. When transported south they knew to head north back to their dens, and when transported north they knew to head south. This showed that not only can lobsters determine a compass heading, but also detect their position relative

to their dens based on nothing other than magnetic cues. These findings are valuable because they show two different animals that are very phylogenetically different from each other but are both capable of true magnetic navigation³.

A third type of animal that was examined in this study was a species of nudibranch (sea slug) that live in the intertidal zones of the ocean. This species has been demonstrated to possess magnetoreceptive capabilities that it uses to navigate the levels of the different intertidal zones so that it doesn't dry out and can find food more efficiently. The reason that the nudibranch makes such a valuable organism for study is because it has a very simplistic central nervous system. Many studies of magnetoreception have looked at either the response that an animal displays, or what mechanism of magnetoreception is most likely at play; but the neurological pathways that are involved in the response would be nearly impossible to study, especially in the complicated network of vertebrate species. Because the nudibranch has such a simple nervous system, it has frequently been used as a test subject for evaluating neural pathways down to the cellular level, so a great body of knowledge already existed for the nudibranch brain at the time of this study, including maps of the known functions of the 7,000 neurons that make up its ganglia. In order to study the cellular components of the magnetoreceptive response, the ganglia of the nudibranch *Tritonia diomedea* were dissected out from the subject and connected to

laboratory instruments that can measure action potentials and the responses were compared to others in the presence of magnetic fields of varying direction and strength. What they found were 3 pairs of neurons that show a response to magnetic fields: the Pd5, Pd6, and Pd7 neurons. The Pd5 and Pd6 neurons were shown to become excited when in the presence of a magnetic field that is changing direction, while the pair known as Pd7 seemed to be inhibited by the same stimulus. The Pd5 and Pd6 neurons were then found to be associated with the function of ciliated cells on the foot of the nudibranch, showing their role in locomotion and bridging the gap between the stimulus and the response. The Pd7 neuron pair bears a strong resemblance to the other two; however, the function of the Pd7 pair is still unknown, although the authors suspect that they may be associated with the anterior sensory structures and play a role in determining and maintaining a directional heading when the nudibranchs are migrating between the zones of the intertidal³.

This study was one of the first to show what magnetoreception looks like from a neurological standpoint, and was during a time when the identity of the magnetoreceptors were still unknown for all animals (today CRY is one of the forerunning candidates for a magnetoreceptor). Based on their findings the authors believe that the receptors, which remain unknown, that are associated with the neurons they looked at only represent a small piece of the overall

sensory pathway, and they suspect that higher-order processing of the magnetoreceptive signal may be an important piece of the mechanism.

In the paper by Alerstam (2006) he makes the argument that magnetoreceptive capabilities are likely to be found in more animals and for more common, everyday tasks than previously thought². In a study by Phillips *et al.* (2013) they ran magnetoreception tests on mice performing a simple task⁶. The basis of the experiment was to use a plus shaped water maze in which only one of the 4 radiating channels had a platform that the mice would be able to climb onto. The maze was placed inside a controlled magnetic field and mice were initially trained by placing them in the maze arm with the platform and letting them stay there for 10 seconds to theoretically learn where the platform was in with respect to the magnetic field. The mice were then taken out and allowed to dry off, and then trained again with the platform and magnetic field each rotated 180° so that they were still the same with respect to each other. The following morning the mice were given their test run by using a device to release them into the middle of the maze, and then a tracking camera recorded their movements through the maze. The average time spent in each arm of the maze was then measured to determine if the mice showed any preference to any arm based on what they learned during their training. Analysis of their data showed that the mice did show a statistically significant response to the magnetic field and the average

time spent in each arm of the maze was greatest in the arm that they had been trained to know had the platform. Based on their results, the authors concluded that the type of mice that they used in this study (C57BL/6 mice) have a well developed magnetic compass sense that they can use to map out a new environment and become more familiar with it. Previous studies have shown that mice use a similar learned magnetic sense when nest building¹⁴, and it is suspected that mice use some level of magnetoreception for many other tasks as well. Unlike migratory animals, this study demonstrated that magnetic fields could be used for more localized, everyday tasks, as hypothesized in Alerstam's paper.

However, the researchers who conducted this experiment also discuss the conditions that are required for the magnetoreceptive response to be shown, and they are somewhat strict. The facility that the experiment was carried out in was specifically designed for tests involving magnetic fields, and it was insulated to the point that it was essentially a building within a building within a building. This was mostly to block out any external noises that the mice could interpret as a directional cue during testing. Despite this, the authors note that some sounds, such as thunder and heavy machinery could still make it through to the testing chamber, and if any such sounds could be heard during a trial, the data for that run would be excluded from the analysis. The room itself was designed so that it

would be symmetrical to eliminate any visual cues, and the water in the maze was colored white so that the mice could not see through it when looking for the platform. One major concern that the authors note is the possibility of olfactory cues left behind by previous mice that might interfere with the experiment, so in between trials they would wash down the sides of the maze and mix the water around so that any possible olfactory cues would be homogenous in the chamber. Unexpected mouse behavior during training and release were also sometimes enough to warrant exclusion of that trial run, for example, if the mouse grabbed onto the side of the wall during training or if the release device bumped into the sides as the mouse entered the maze then the data from that run would be thrown out. While it is good practice to be thorough and conscientious about what possible factors could influence the outcomes of an experiment, it can sometimes weaken the credibility of the results as more and more data points are thrown out. While testing an animal for something subtle like a magnetoreceptive response does call for a more isolated environment, other studies have seemed to take this as a liberty to throw out any data points that were different from what they were expecting, such as suspected interference caused by anything from fluorescent lights, cell phones, or even just bumping into the coils of the magnetic field generator. So while this study does

seem to show a clear response to the magnetic field, the results would hold more weight had they not thrown out as many data points.

When discussing the different proposed mechanisms of magnetoreception (magnetite model vs. radical pair model), it is important to determine the capabilities and usefulness of each. In a paper by Frings (2009), he writes about the mechanisms by which many different sensory processes take place, from photoreceptors, to mechanoreceptors, to possible magnetoreceptors⁴. When discussing the ability to detect magnetic fields, he discusses both the magnetite and radical pair mechanisms, and the types of data each would theoretically be able to gain from a magnetic field. There are three main data points that can be gained from a magnetic field: the inclination of the field relative to earth, the direction of magnetic north, and the intensity of the field. The two magnetoreception models have different capabilities of detecting these different parameters, meaning that certain information may be gained by use of the radical pair mechanism, while other information can only be gained by the magnetite mechanism. Based on the molecular mechanisms of the two models, the radical pair model would likely be more useful for gaining information about the direction of the magnetic field and maintaining a compass heading. This hypothesis is supported by the suspected use of the radical pair model in migratory birds. The magnetite model on the other hand is thought to be more

useful when determining positional information such as longitude and latitude because the magnetite model is sensitive to the isoclinics and isodynamics of the earth's magnetic field. Understanding the uses of these two different models is important because it can help to determine which model is more likely to be present in a particular organism.

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