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4
5 Using artificial selection to assay magnetic field orientation in *Drosophila*

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3

4 **Abstract**

5 A phylogenetically diverse group of animals can detect and orient to Earth-strength magnetic
6 fields, yet the neural circuitry underlying magnetoreception has not been identified in any
7 animal. A model organism with a tractable genome, an accessible nervous system, and a robust
8 behavioral response to Earth-strength magnetic fields would facilitate the identification of a
9 magnetoreceptor and the related neural circuitry. Experimental evidence suggests that
10 *Drosophila melanogaster* are able to orient to magnetic fields using a light-dependent
11 magnetoreception mechanism, but the orientation behaviors have not been independently
12 replicated. Moreover, the mechanisms of magnetoreception have not been investigated for other
13 dipterans. There is evidence that lepidopterans and hymenopterans that respond to magnetic
14 fields have either magnetite-based magnetoreception or both light-dependent and magnetite-
15 based mechanisms, however an exact magnetoreceptor is not known and the neural circuitry is
16 not well studied. To study a genetically-tractable organism with a robust north-seeking or south-
17 seeking behavior, we used a sequential Y-maze to identify *Drosophila melanogaster* with
18 directional preferences and performed 15 generations of artificial selection. We also performed
19 parallel experiments to select for phototaxis as a positive control. Using wild-caught *Drosophila*
20 *melanogaster*, we were able to decrease positive phototaxis of the flies over 15 generations, but
21 we were unable to create flies with a clear northward or southward orientation preference. We
22 found no convincing evidence for an innate magnetic-field orientation behavior in male or
23 female flies.

24

1 **Key words:** magnetoreception, selection, orientation, navigation, *Drosophila melanogaster*

2

3 **Introduction**

4 The Earth's magnetic field is a ubiquitous and reliable orientation cue (Skiles, 1985). As such, it
5 is not surprising that a wide diversity of animals have been shown to detect Earth-strength
6 magnetic fields, including sea slugs, bees, lobsters, fish, reptiles, birds, and mammals (Cain *et*
7 *al.*, 2005; Hellinger and Hoffman, 2012; Mouritsen and Hore, 2012; Begall *et al.*, 2014;
8 Lambinet *et al.*, 2017). Yet while magnetoreception is widespread in the animal kingdom,
9 magnetic field orientation behavior can be difficult to detect and study in animals. For example,
10 for newts to demonstrate orientation to Earth-strength magnetic fields, the experimental surface
11 cannot have elevation changes greater than 0.1° (Phillips and Boreland, 1994). Magnetic
12 orientation in dogs is disrupted by fluctuations in the magnetic field declination as small as
13 0.001° per hour (Hart *et al.*, 2013). Similarly, magnetic orientation in amphipods is eliminated in
14 the presence of radiofrequency interference that is less than 0.1% of the strength of the Earth's
15 magnetic field (Tomanova and Vacha, 2016).

16

17 Despite the challenges of studying magnetic orientation behavior and the lack of an identified
18 magnetoreceptor, two different mechanisms for detecting magnetic fields have been identified: a
19 magnetite-based mechanism and a light-dependent mechanism, involving a Radical Pair-Model
20 of electrons in photopigments. Evidence for both of these mechanisms has been found within
21 arthropods. Magnetic pulse experiments and experiments performed in darkness show that ants,
22 bees, butterflies, and spiny lobsters use a magnetite-based mechanism to orient to Earth-strength
23 magnetic fields (Schmitt and Esch, 1993; LaRue *et al.*, 2006; Riveros *et al.*, 2014; Ernst and

1 Lohmann, 2016). Experiments with different wavelengths of light show that bees, butterflies, and
2 mealworms use a light-dependent magnetic field orientation mechanism (Leucht, 1984; Vacha *et*
3 *al.*, 2008; Guerra *et al.*, 2014). Additionally, behavioral wild-type and transgenic experiments
4 indicate that *Drosophila melanogaster* (Meigen, 1830) have a light-dependent mechanism of
5 magnetoreception that uses Cry photoreceptors (Phillips and Sayeed, 1993; Dommer *et al.*, 2008;
6 Gegear *et al.*, 2010). Nonetheless, the identification of a magnetoreceptor and an accompanying
7 neural circuit underlying magnetoreception has been frustratingly elusive (Nordmann *et al.*,
8 2017).

9
10 In order to facilitate the identification of a magnetoreceptor, model organisms for
11 magnetoreception studies should have a tractable genome, an accessible nervous system, and a
12 robust behavioral response to Earth-strength magnetic fields (25-65 μ T) (Nordmann *et al.*, 2017).
13 A robust behavior is necessary so that studies can be replicated in order to independently verify
14 and build on previous findings. Because it is already a model organism for genetics and its
15 nervous system is extensively studied, *Drosophila melanogaster* is an attractive species for
16 investigating the neural circuitry of magnetoreception. However, the magnetic orientation
17 behavior of *D. melanogaster* is not consistent across studies. For example, an early study
18 indicated that only adult male flies orient to magnetic fields (Phillips and Sayeed, 1993), while a
19 subsequent study showed that both males and females orient to magnetic fields (Gegear *et al.*,
20 2008). Similarly, one study showed that larval *D. melanogaster* could be trained to orient to
21 magnetic fields (Dommer *et al.*, 2008) but did not have an innate magnetic orientation
22 preference, while another study showed that larvae, as a group, have an innate quadrimodal
23 distribution (Painter *et al.*, 2013).

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While evidence indicates that *Drosophila melanogaster* have a light-dependent mechanism for magnetoreception, the lepidopterans and hymenopterans that respond to magnetic fields appear to have either magnetite-based methods (Chittka *et al.*, 1999; Camlitepe *et al.*, 2005; Riveros *et al.*, 2014; Anderson and Vander Meer, 1993) or both light-dependent and magnetite-based mechanisms (Liang *et al.*, 2016; Leucht, 1984; Perez *et al.*, 1999; Guerra *et al.*, 2014); therefore, it is plausible that *D. melanogaster* also have a magnetite-based magnetoreceptor. We used a Y-maze and artificial selection to attempt to create a population of *Drosophila melanogaster* with a robust, magnetic-field orientation behavior. We performed a parallel experiment using directional light cues for phototaxis as a positive control. We were able to alter phototactic behavior but did not find convincing evidence for magnetic orientation behavior.

Methods

ANIMAL CARE

A wild population of *Drosophila melanogaster* (Generation 0), was collected from a composting site in Monmouth, OR, USA, and were passaged for at least 10 generations before experiments were run. Wild-caught flies were used in order to avoid dampened behavioral responses that can occur in flies raised in the lab for many years (Russell and Kurtz, 2012). These could obscure harder to detect behaviors such as magnetic orientation. Flies were maintained in a 12h:12h light:dark cycle at 25°C on standard cornmeal-dextrose medium supplemented with 0.1% Nipagen (p-hydroxybenzoic acid methyl ester; Sigma-Aldrich, St. Louis, MO) to inhibit mold.

MAZE DESIGN AND ARTIFICIAL SELECTION

1 In order to artificially select flies with a specific directional preference, we built a horizontal
2 progressive Y-maze with 10 sequential choices which allowed the flies to go right or left at each
3 choice-point based on environmental cues (Figure 1; James *et al*, 2016). The Y-maze was made
4 out of transparent Tygon tubing with an inner diameter of 3/16" and 1/8" inner diameter Y-type
5 transparent polypropylene connectors. To prevent flies from back-tracking once a decision was
6 made, transparent plastic pipette tips were cut and inserted into the Y-connectors and the vials at
7 the beginning and ends of the maze were fitted with foam stoppers punctured by the cut pipette
8 tips. Transparent collection vials were filled with food to encourage flies to finish the maze and
9 to maintain the flies until they were counted the next day. The starting vial did not contain any
10 food and was covered with aluminum foil to block light and encourage flies to exit the vial into
11 the maze.

12
13 The maze was illuminated with full spectrum visible light (400-700 nm) produced by two desk
14 lamps with 40 W incandescent light bulbs placed on the same surface as the maze, directed
15 toward the ceiling to create the ambient light for each run. A plastic light diffuser was placed
16 over the top of the maze to ensure a smooth light gradient. The light spectrum was measured
17 with a Wabash Instrument Corporation SP125 Spectrophotometer (Wabash, IN) after the light
18 had passed through the diffuser.

19
20 The maze was oriented so that a choice to enter the right branch of a Y-connector represented
21 either a northward or southward choice, depending on the trial. If flies have a directional choice
22 in the maze, that choice could be due to a number of factors including the magnetic field, a right-
23 turning or left-turning preference of the flies, or a bias in the maze design. To account for a right-

1 left preference in the flies or a bias in the maze, the maze orientation was altered randomly so
2 some weeks north was to the right and other weeks north was to the left. Maze orientation was
3 determined using a random number generator in Microsoft Excel (Microsoft Corporation,
4 Redmond, WA).

5
6 The strength of the magnetic field in Monmouth, OR, is approximately 52 μT (NOAA National
7 Centers for Environmental Information). Using the cellular phone app Magneto (Caffeina,
8 Parma, Italy), we measured the intensity of the natural field in Monmouth, OR, as 48 μT . This is
9 similar to the NOAA measurements, however to confirm our measurement we used a second
10 cellular phone app Magnitude (Ramesh Babu), which measured the intensity as 50 μT . The
11 benefit of using a cellular phone app is that it is inexpensive and makes this type of research
12 more accessible. The average ambient magnetic field in the room where we performed the
13 artificial selection experiments was $44 \pm 1 \mu\text{T}$ (mean \pm s.e.m.). The average orientation of the
14 transverse (i.e., left-right) axis was $29^\circ \pm 8^\circ$ (\pm s.e.m.) to the east of due north. The 95%
15 confidence interval was $14^\circ - 44^\circ$, which overlaps with the reported innate orientation preference
16 of *D. melanogaster* larvae ($41^\circ - 44^\circ$; Painter *et al*, 2013). We performed a replicate experiment in
17 an alternative room which had a mean magnetic field strength of $28 \pm 2 \mu\text{T}$. In this room, the
18 average orientation of the transverse axis of the maze was $20^\circ \pm 11^\circ$ to the east of due north, with
19 a 95% confidence interval of $358^\circ - 42^\circ$.

20
21 *D. melanogaster* were given 24 hours to complete the maze. After the Generation 0 flies
22 completed the maze, the 20% of flies collected from the northernmost vials were interbred to

1 create a “North” population of flies; while, the 20% of flies collected from the southernmost
2 vials were interbred to create a “South” population of flies. For subsequent generations, after the
3 North flies completed the maze, the 20% of flies in the northernmost vials were used to create
4 the next generation. Likewise, after the South flies completed the maze, the top 20% of south-
5 seeking flies were used to create the next generation for the South population. After each run
6 through the maze, we anesthetized flies with CO₂ to count the number of male and female flies in
7 each vial. Between runs, we allowed 2-3 weeks for adequate breeding of each generation. During
8 off-weeks when flies were breeding, the maze was flushed with tap water and allowed to air dry
9 until the next use.

10

11 Concurrently with the magnetic field orientation experiment, we performed a parallel experiment
12 to select for positive or negative phototaxis. We used one 40 W desk lamp with full-spectrum
13 light and placed a plastic light diffuser in front of the light to ensure a smooth light gradient from
14 light to dark across the maze. The maze was oriented so a choice to enter the right branch of a Y-
15 connector represented either a light or dark choice, depending on the trial; maze orientation was
16 determined pseudorandomly so some weeks light was to the right and other weeks light was to
17 the left.

18

19 TESTS FOR MAGNETIC FIELD ORIENTATION PREFERENCES

20 After 15 generations of artificial selection, we performed 10 replicate trials in the north-south
21 maze conditions for each population (Generation 0, Generation 15 North, and Generation 15
22 South flies). Independent pools of different flies were used for each trial. During these trials, the
23 orientation of the maze (i.e., whether north was to the right or to the left) was determined

1 pseudorandomly by using a random number generator to determine maze orientation until one
2 orientation was used 5 times. An equal number of trials were run with each orientation. We used
3 a similar method to pseudorandomly determine which population would be used on any given
4 day. We performed 10 replicates in the light-dark maze conditions, as well, using the same
5 methods of pseudorandomization. These experiments, as well as the artificial selection maze
6 runs, were all performed blind; the researcher who set-up the maze and the researcher counting
7 the flies at the end of the maze did not know which strain of flies with which they were working.

8

9 As described in the results, our initial data indicated that *D. melanogaster* might have an innate
10 preference for north. Therefore, we performed additional experiments in an attempt to confirm
11 this finding. We performed 30 replicate N-S maze trials (15 with north to the right, 15 with north
12 to the south) with the Generation 0 flies in an alternative room. Independent pools of different
13 flies were used for each trial. The orientation of the maze was again determined
14 pseudorandomly. Since all flies were from the Generation 0 population, we could not blind the
15 researchers to the fly population; however, we labeled the north-south compass axes 'X' and 'Y'
16 (rather than 'N' and 'S') so the researchers performing the experiments did not know which side
17 of the maze was north and which side was south.

18

19 We also performed 20 replicate N-S maze trials (10 trials with north to the right, 10 trials with
20 north to the south) with the Generation 0 flies in the original room but with a newly constructed
21 maze and with a Faraday cage surrounding the maze to block potential electromagnetic
22 disruption. Independent pools of different flies were used for each trial. The orientation of the
23 maze was again determined pseudorandomly.

1

2 Over the course of all experiments, 33 trials were discarded due to errors in setting up the maze
3 or problems that occurred during the experiment. Examples of errors in setting up the maze
4 included setting up the maze in the wrong orientation (4 trials), leaving the wrong combination
5 of lights on in the room (3 trials), and using a vial containing food as the start vial (3 trials).
6 Examples of problems that occurred during the experiment included breaks in the connectors
7 joining the maze together (5 trials), a fly getting stuck in a pipette tip and blocking the maze (4
8 trials), and a light bulb dying during the experiment (3 trials). In 5 cases the majority of flies did
9 not leave the start vial for unknown reasons. Some of the criteria for excluding maze trials were
10 pre-determined, such as errors in setting up the maze. Other criteria were not foreseen, such as
11 flies becoming stuck in the maze; however, those criteria were applied consistently.

12

13 All statistical analyses were performed with IBM SPSS Statistics 24 (IBM Corporation, Armonk,
14 New York).

15

16 **Results**

17 ARTIFICIAL SELECTION

18 If flies choose randomly at each branch point of the maze, we would expect that they would exit
19 the maze, on average, in vial 5. Our first maze trial with Generation 0 flies suggested that the
20 flies had a preference for light ($n = 98$ flies; vial 7.3 ± 0.2 ; mean \pm s.e.m.; Figure 2A) and for the
21 north side of the maze ($n = 148$ flies; vial 5.8 ± 0.2 ; Figure 2B). However, because the flies were
22 released in the maze as a group, we were concerned that their behavior in the maze was not
23 independent. If flies were independent, we would expect that flies would exit the maze in a

1 normal distribution around the average numbered exit vial. We found that the distribution of flies
2 in the collection vials was different from a normal distribution for both the light-dark trials
3 (Pearson chi-square, $p < 0.001$) and the north-south trials ($p < 0.001$). In other words, the flies
4 did not move through the maze independently of each other (Figure 2). Any statistical analyses
5 on individual flies in runs through the maze to determine orientation behavior would be
6 pseudoreplication. Thus we considered the result of each run through the maze as a single data
7 point.

8

9 During the 15 generations of artificial selection, the light-dark trials had an average of 123 ± 14
10 (\pm s.e.m.) flies per trial, while the north-south trials had an average of 136 ± 13 flies per trial.

11 The average number of “light” choices made by the light-selected and dark-selected fly
12 populations appeared to diverge during the course of our artificial selection protocol (Figure 3A);
13 in contrast, the average number of “north” choices made by the north-selected and south-selected
14 fly populations did not appear to differ (Figure 3B). Data for the first 12 generations of selection
15 were previously published (James *et al.*, 2016).

16

17 After completing 15 generations of selection, we first analyzed our data to establish whether flies
18 had a rightward or leftward-bias, either due to innate behavioral preferences or due to a structural
19 bias in the maze. The flies were scored based on their final collection vial, with the rightmost
20 vial representing 10 right choices and the leftmost vial representing 0 right choices. For both the
21 light-dark trials and the north-south trials, the flies had a slight leftward preference (vial 4.3 ± 0.2
22 and vial 4.2 ± 0.1 , respectively). The leftward preference, however, was not different across

1 populations for either the light-dark trials (ANOVA, $F(2,54) = 0.94$, $p = 0.40$; Figure 4) or the
2 north-south trials (ANOVA, $F(2,54) = 0.22$, $p = 0.81$).

3

4 For light-dark trials, orientation with respect to light was different across the fly populations
5 (ANOVA, $F(2,54) = 3.84$, $p = 0.03$; Figure 5), but was not affected by the sex of the flies
6 ($F(1,54) = 2.13$, $p = 0.15$; data not shown). For both sexes of each population of flies, the flies
7 oriented toward the light relative to the mean vial; the average score for Generation 0 flies ($1.8 \pm$
8 0.2 vials to the light side of mean vial) was significantly different than the average score for the
9 dark-selected flies (1.1 ± 0.2 vials to the light side of mean vial; post-hoc tukey test, $p = 0.04$;
10 Figure 5).

11

12 For north-south trials, orientation was not different across the fly populations (ANOVA, $F(2,54)$
13 $= 1.14$, $p = 0.33$), and was not affected by the sex of the flies ($F(1,54) = 0.79$, $p = 0.38$). For all
14 populations of flies tested in the north-south maze conditions, the flies oriented to the north of
15 the mean vial (pooled mean = 0.5 ± 0.1 vials to the north of mean vial, no significant difference
16 found).

17

18 When the flies were scored based on their number of choices to go right, the number of right
19 choices was significantly different when the light was on the right (vial 5.8 ± 0.2) compared to
20 when the light was on the left (vial 2.8 ± 0.3 ; ANOVA, $F(1,24) = 88.69$, $p < 0.001$; Figure 6A).
21 However, when analyzed in this manner, there was not a difference between fly populations
22 ($F(2,24) = 2.64$, $p = 0.09$), nor was there an interaction between the location of the light (right or

1 left) and the fly populations (ANOVA, $F(2,24) = 2.94$, $p = 0.07$), although both approached
2 statistical significance.

3

4 When the flies were scored based on their number of choices to go right, the number of right
5 choices was significantly different when north was to the right (vial 4.8 ± 0.2) compared when
6 north was to the left (vial 3.7 ± 0.2 ; ANOVA, $F(1,24) = 11.91$, $p = 0.002$; Figure 6B). There was
7 not a difference between fly populations ($F(2,24) = 0.08$, $p = 0.92$), nor was there an interaction
8 between the direction of north (right or left) and the fly populations (ANOVA, $F(2,24) = 0.64$, p
9 $= 0.54$).

10

11 REPLICATION USING ORIGINAL FLY POPULATIONS

12 In order to determine if *D. melanogaster* had a northward preference because they were orienting
13 to the magnetic field or because there was an unknown variable in the room (e.g., a temperature
14 gradient or a humidity gradient), we repeated the experiment with the Generation 0 flies in
15 another room. The average number of right choices was not significantly different (t-test (equal
16 variance), $p = 0.29$) when north was to the right (vial 4.7 ± 0.1) compared to when north was to
17 the left (vial 4.4 ± 0.2 ; Figure 6B). In the new room, the flies still had a leftward bias in the maze
18 (vial 4.5 ± 0.1) that was not significantly different from the leftward bias observed in the original
19 room used for the north-south trials (t-test (unequal variance), $p = 0.16$).

20

21 When we used a new maze surrounded by a Faraday cage, but tested in the original experimental
22 room, we found that the average number of right choices was not significantly different (t-test
23 (equal variance), $p = 0.75$) when north was to the right (vial 5.1 ± 0.2) compared to north was to

1 the left (vial 5.2 ± 0.2 ; Figure 6B). The flies did not have a leftward bias in the new maze with a
2 Faraday cage (vial 5.0 ± 0.1); this value was significantly different from the leftward bias
3 observed in the original maze (t-test (unequal variance), $p = 0.002$).

4 5 **Discussion**

6 While a number of studies have shown that *Drosophila melanogaster* can detect and orient to
7 magnetic fields, the results are not consistent across studies. An early study showed that when
8 trained to associate 365 nm wavelength light with a compass direction, adult males oriented to
9 magnetic fields while adult females did not (Phillips and Sayeed, 1993). Adult males also
10 oriented successfully under 500 nm wavelength light, although their orientation was altered by
11 90° . Subsequent studies with adult *D. melanogaster* found that both males and females can learn
12 to orient towards a magnetic field that is 10-times stronger than Earth-strength magnetic fields in
13 the presence of <420 nm wavelength light, but not in the presence of 500 nm wavelength light
14 (Gegear *et al.*, 2008). A recent study also demonstrated that both male and female adult
15 *Drosophila* can be trained to orient to a food source using Earth-strength magnetic fields under
16 broad-spectrum light, but the effect of different wavelengths was not tested (Lee *et al.*, 2018);
17 Studies with *Drosophila melanogaster* larvae also indicate that both males and females can
18 orient to Earth-strength magnetic fields, however the effect of 500 nm light on orientation was
19 either not tested or was tested but not reported (Dommer *et al.*, 2008; Painter, *et al.*, 2013).

20
21 In an effort to establish how *Drosophila melanogaster* detect magnetic fields, Gegear *et al.*
22 (2008; 2010) created transgenic flies with a mutant *ultraviolet-A/blue light photoreceptor*
23 *cryptochrome* gene. Without functional cryptochrome (Cry), adult flies were unable to orient to a

1 learned magnetic field that was 10-times stronger than Earth-strength magnetic fields. Additional
2 studies examining the role of *Cry* in *Drosophila* magnetoreception have used behavioral assays
3 that are not related to magnetic orientation (circadian rhythms, geotaxis, mating behaviors, and
4 seizures) or used magnetic fields that were 2-1000 times stronger than Earth-strength magnetic
5 fields (Yoshii *et al.*, 2009; Fedele *et al.*, 2014a; Marley *et al.*, 2014; Wu *et al.*, 2016). Subsequent
6 studies on circadian rhythms and geotaxis have found similar results using magnetic fields that
7 are similar in strength to the Earth's magnetic field (Fedele *et al.*, 2014b; Bae *et al.*, 2016). These
8 studies show that *Cry* functioning can be disrupted by electromagnetic fields, but most do not
9 provide evidence that *Drosophila* have the ability to detect Earth-strength magnetic fields using
10 *Cry* and translate that into directional information. Magnetic fields could act as *Cry* agonists or
11 antagonists, thus for *Cry* to be positively identified as a magnetoreceptor, or part of a
12 magnetoreceptor, animals need to be able to gather useful information from the interaction
13 between *Cry* and Earth-strength magnetic fields, and this has not been demonstrated.

14
15 If the ultimate goal is to determine how, at a cellular level, *Drosophila* use magnetic field
16 information to guide behavioral patterns, then we need to use a reliable magnetic orientation
17 assay that is sensitive to Earth-strength magnetic fields and produces a relevant behavioral
18 response. In this study, we devised a simple method for evaluating magnetic orientation ability
19 and attempted to use artificial selection to create populations with robust magnetic field
20 orientation behaviors. For two primary reasons we chose not to use a Faraday cage in our
21 selection experiments to block RF interference even though RF fields with a strength of 0.002
22 μT have been shown to disrupt magnetic field orientation behavior (Tomanova and Vacha,
23 2017). First, although experimental evidence indicates that *Drosophila* have a light-dependent

1 mechanism for detecting magnetic fields, the most closely related insects with magnetic
2 orientation behavior use either magnetite-based magnetoreception or both magnetite-based and
3 light-dependent magnetoreception, and RF fields should not affect magnetite-based
4 magnetoreception (Chittka *et al.*, 1999; Camlitepe *et al.*, 2005; Riveros *et al.*, 2014; Anderson
5 and Vander Meer, 1993; Liang *et al.*, 2016; Leucht, 1984; Perez *et al.*, 1999; Guerra *et al.*,
6 2014). Second, we wanted to keep the experimental design as straightforward as possible to
7 increase the probability of successfully replicating experiments. For this reason, we also used
8 freely available cell phone applications to measure the magnetic field. Researchers at any
9 institution have access to these applications, but may not have access to a magnetometer.

10

11 During our first trials, we found that our flies did not move independently in the maze (Figure 2).
12 *Drosophila* are known to follow each other, particularly when males are courting females (Sakai
13 *et al.*, 1997); thus, this result is not surprising. Over the course of 15 generations of selection, the
14 strength of the positive phototaxis of our light-selected and dark-selected flies appeared to
15 diverge (Figure 3A), but we did not perform statistical tests on individual maze trials because the
16 flies were not independent within a trial. Replicate trials with our original Generation 0 flies,
17 Generation 15 light-selected and Generation 15 dark-selected showed that the dark-selected flies
18 had a significantly less positive phototaxis than the original population (Figure 5). The light-
19 selected flies were not different from the original population, likely due to a ceiling effect on
20 selection. Using a lab strain of fruit flies, Hadler (1964) was able to select for both positive
21 phototaxis and negative phototaxis because his beginning population of lab-bred flies had a
22 much less positive phototaxis than our original wild-caught population. The difference in the
23 original phototactic behavior in this study compared to Hadler (1964) is not surprising given

1 similar differences when wild-caught *Drosophila melanogaster* are directly compared to lab
2 strains of *D. melanogaster* (Russell and Kurtz, 2012).

3

4 In contrast to the light selection experiments, selection for magnetic field-based directional
5 preference did not create any differences between the populations of flies (Figure 5). We did find
6 evidence, however, of an innate northward preference of the flies in the maze (Figure 6). While
7 we could not identify any structural elements of the room that would affect the orientation of the
8 flies, it was possible that there was a temperature gradient, humidity gradient, light gradient, or
9 other factors affecting the orientation of the flies; therefore, we repeated the experiment with
10 Generation 0 flies in an alternate room. In the new room, there was no obvious magnetic field
11 orientation behavior of the flies. We conclude that our evidence does not support the hypothesis
12 that fruit flies orient to Earth-strength magnetic fields using a magnetite-based mechanism. It is
13 worth noting that, compared to the second experimental room where the flies did not have a
14 north-south preference, the original experimental room had a stronger magnetic field which was
15 also more similar to the local magnetic field. This leaves the door open to the possibility that
16 raising flies in a room with a particular magnetic field intensity allows them to only show
17 magnetic orientation behavior in an assay room of similar measurements, as has been seen in
18 birds (Winklhofer *et al.*, 2013), or that our flies do show an innate north preference, but it is
19 obscured in our second room.

20

21 In a final experiment, we also performed replicate experiments in the original room while using a
22 Faraday cage to block RF fields to determine if adult *Drosophila melanogaster* have an innate

1 north-south directional preference. We did not find evidence of an innate orientation behavior in
2 male or female flies.

3
4 Magnetoreception has been an active area of research for over 50 years (Nordmann *et al.*, 2017),
5 yet our understanding of magnetoreception and the accompanying receptors has lagged behind
6 other sensory receptors. There are many possible explanations for why the identification of a
7 magnetoreceptor has been difficult, but one reason is that researchers have not focused on a
8 model organism with a reliable magnetic orientation assay and an accessible nervous system that
9 can be easily modified genetically (Nordmann *et al.*, 2017). While we failed to produce fruit flies
10 with a magnetic orientation behavior in this study, if *D. melanogaster* can navigate using Earth-
11 strength magnetic fields, then our strategy of artificial selection should be able to produce a
12 robust orientation behavior for investigating light-dependent magnetoreception in *Drosophila*.

14 **Conflict of Interest**

15 The authors declare that they have no conflict of interest.

17 **Data availability**

18 Our data has been made available at figshare.com (DOI: 10.6084/m9.figshare.5995420,
19 <https://figshare.com/s/1662d8e20c85709342cc>).

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1 **Figure legends**

2

3 **Figure 1: Schematic diagram of the sequential Y-maze.** The figure depicts one of the maze
4 conditions, where ten right choices results in a fly ending the maze in vial 10, and right choices
5 are to the north. For other trials, south was to the right. For light/dark trials, for some trials the
6 light source was on the right side of the maze and for other trials the light source was on the left
7 side of the maze. The location of the light was changed by rotating the maze horizontally rather
8 than by moving the light.

9

10 **Figure 2: Predicted distribution and actual distributions of flies in the collection vials for**
11 **Generation 0 flies.** (A) The first light-dark maze trial with Generation 0 flies ($n = 98$) resulted in
12 flies exiting the maze with a mean vial number of 7.3 ± 0.2 (\pm s.e.m.). The actual distribution
13 was significantly different than the expected normal distribution for a mean of 7.3 (Pearson chi-
14 square: $p < 0.001$). (B) The first north-south maze trial with Generation 0 flies ($n = 148$) resulted
15 in flies exiting the maze with a mean vial number of 5.8 ± 0.2 . The actual distribution was
16 significantly different than the expected normal distribution for a mean of vial 5.8 ($p < 0.001$).

17

18 **Figure 3: Average number of times flies chose the (A) light branch or (B) north branch of a**
19 **Y-junction while moving through the maze over fourteen generations of artificial selection.**
20 (A) Selection for positive or negative phototaxis. There were an average of 123 ± 14 (\pm s.e.m.)
21 flies per trial. (B) Selection for north or south directional preference. There were an average of
22 136 ± 13 flies per trial. Trendlines are linear regression lines anchored at the average score for
23 Generation 0 flies. Error bars represent s.e.m. across trials.

24

1 **Figure 4: Average number of times flies chose the right branch of a Y-junction.** In light-dark
2 trials, the light was on the right side of the maze for half of the trials and on the left side of the
3 maze for the other half of the trials (n = 10 trials for each population). In north-south trials, north
4 was toward the right for half of the trials and toward the left for half of the trials (n = 10 trials for
5 each population). In the absence of a right-left preference by the flies in the maze, the average
6 number of right choices would be 5.0, indicated by the black line. Gen 0 flies are the original
7 population of flies. Gen 15 flies have undergone 15 rounds of artificial selection for positive
8 phototaxis (L), negative phototaxis (D), northward orientation (N), or southward orientation (S).
9 The average number of right choices was not significantly different across fly populations
10 (ANOVA: Light-dark, p = 0.40; North-south, p = 0.81).

11

12 **Figure 5: Average deviation from the expected vial if flies did not express a behavioral**
13 **response to light or to magnetic fields.** For the light-dark trials, a positive value is positive
14 phototaxis. The distance from the mean vial was different across populations (ANOVA, p =
15 0.03; n = 10 trial for all populations). For the north-south trials, a positive value is preference for
16 the north side of the maze. The distance from the mean vial was not different across populations
17 (ANOVA, p = 0.33; n = 10 trials for all populations). P-values shown above bars are for post-hoc
18 Tukey tests. Error bars represent s.e.m. across trials.

19

20 **Figure 6: Average number of right choices under different maze conditions.** (A) Average
21 number of right choices in light-dark trials. The number of choices to go right was significantly
22 different when the light was on the right side of the maze compared to the left side of the maze
23 (ANOVA; p < 0.001; n = 5 trials for each treatment combination). There was no difference

1 between populations. (B) Average number of right choices in north-south trials. The number of
2 choices to go right was significantly different when north was toward the right side of the maze
3 compared to when north was toward the left side of the maze (ANOVA; $p = 0.002$; $n = 5$ trials
4 for each treatment combination). There was no difference between populations. When we
5 repeated the north-south maze conditions in a different room, there was no difference between
6 the orientations of the flies when north was towards the right compared to the left (t-test: $p =$
7 0.29 ; $n = 15$ trials for each maze orientation). When we repeated the north-south maze conditions
8 in the original room, but with a newly constructed maze and a Faraday cage over the maze, there
9 was no difference between the orientations of the flies when north was towards the right
10 compared to the left (t-test: $p = 0.75$; $n = 10$ trials for each maze orientation).

Figure 1

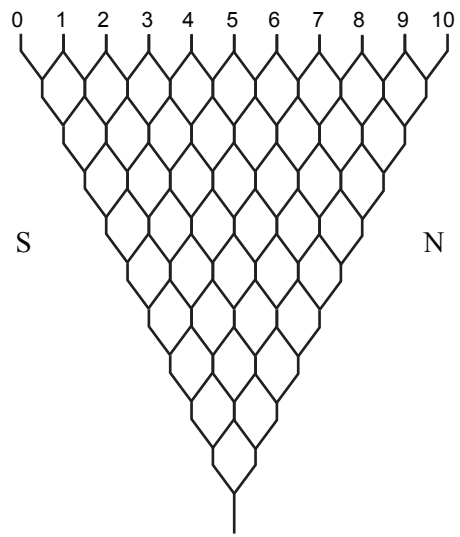


Figure 2

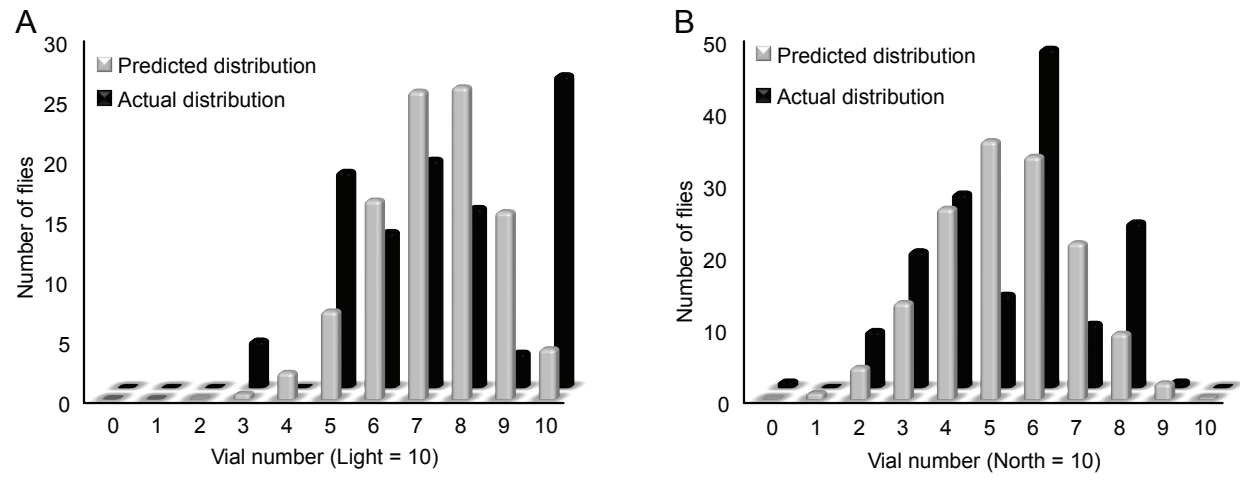


Figure 3

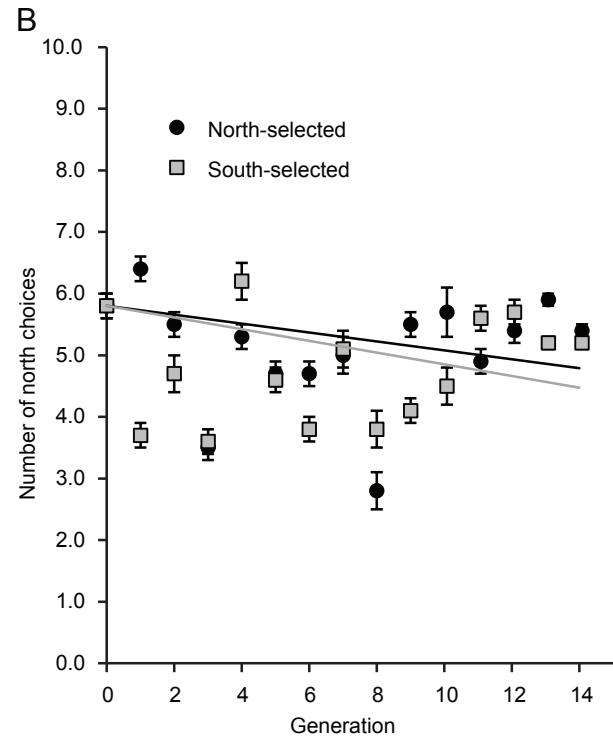
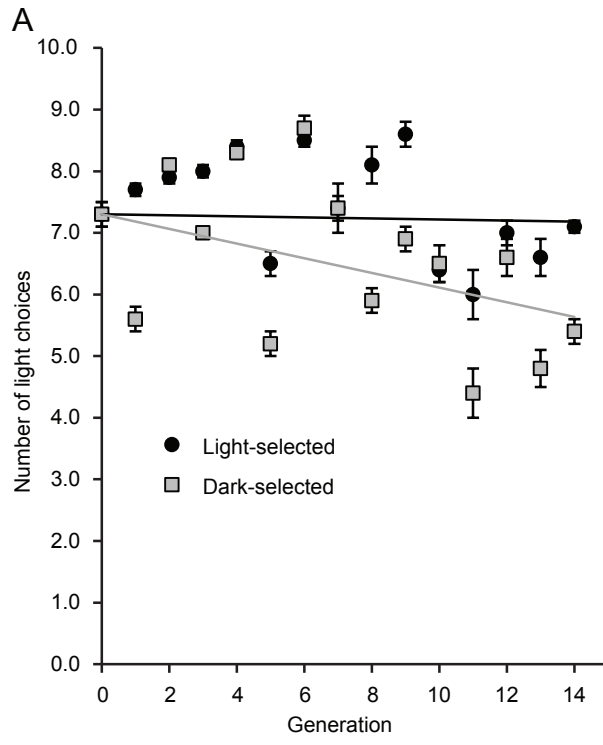


Figure 4

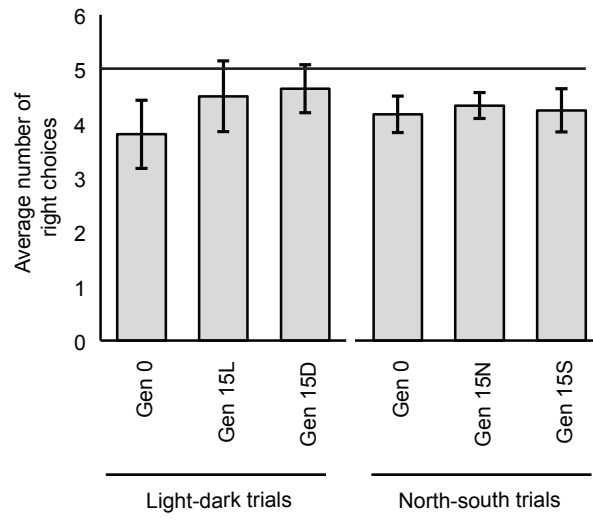


Figure 5

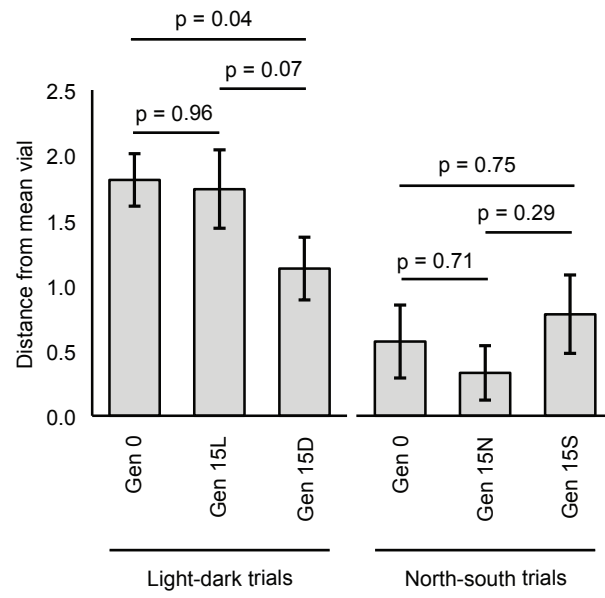


Figure 6

