



Factors that influence magnetic orientation in *Caenorhabditis elegans*

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Abstract

Magnetoreceptive animals orient to the earth's magnetic field at angles that change depending on temporal, spatial, and environmental factors such as season, climate, and position within the geomagnetic field. How magnetic migratory preference changes in response to internal or external stimuli is not understood. We previously found that *Caenorhabditis elegans* orients to magnetic fields favoring migrations in one of two opposite directions. Here we present new data from our labs together with replication by an independent lab to test how temporal, spatial, and environmental factors influence the unique spatiotemporal trajectory that worms make during magnetotaxis. We found that worms gradually change their average preferred angle of orientation by ~180° to the magnetic field during the course of a 90-min assay. Moreover, we found that the wild-type N2 strain prefers to orient towards the left side of a north-facing up, disc-shaped magnet. Lastly, similar to some other behaviors in *C. elegans*, we found that magnetic orientation may be more robust in dry conditions (<50% RH). Our findings help explain why *C. elegans* accumulates with distinct patterns during different periods and in differently shaped magnetic fields. These results provide a tractable system to investigate the behavioral genetic basis of state-dependent magnetic orientation.

Keywords Magnetic orientation · State dependence · Nematode · Migration · Humidity

Abbreviations

NGM Nematode growth medium
AFD AFD sensory neuron pair

Introduction

Like the human use of a compass, organisms that possess a magnetic sense do not simply orient and migrate blindly towards magnetic north; instead, they move at different angles with respect to the magnetic field to guide themselves to their desired destination with the help of other available cues. Desired destinations may satisfy requirements for food, mating, or permissive climate, and thus depend on internal and external factors. These may include the current hunger or disease state of the organism, the time of year, or even life stage (Guerra et al. 2014). Thus, the direction that an organism prefers to orient with respect to a magnetic field will necessarily change to reflect changing needs (Wiltschko and Wiltschko 1996). For example, every spring newly eclosed Bogong moths migrate over 1000 km southward to cooler Australian Alps where they enter a summer dormancy (Warrant et al. 2016). The same moths migrate northward in the fall to return to low-lying plains to breed. To accomplish these long-distance migrations, the Bogong moth appears to process a combination of visual and magnetic cues (Dreyer et al. 2018). When assayed semi-restrained outdoors, the moths show preference for different directions with respect to the magnetic field that correlate with their natural migratory directions (Dreyer et al.

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2018). Turtles and salmon appear to consult a ‘magnetic map’ that is imprinted from birth or inherited, respectively, to swim with respect to the magnetic field towards the goal appropriate for their life stage and season (Putman et al. 2012, 2014).

Mirroring other animals, we found that the nematode *Caenorhabditis elegans* migrated in different preferred directions when orienting to magnetic fields from the earth and artificial sources, and that these directions appeared to depend on several factors (Vidal-Gadea et al. 2015). One factor appears to be time. We obtained opposite results when worms were removed and tested immediately or if tested after spending approximately 30 min in liquid medium following removal from their culture plate. For instance, the wild-type lab strain N2 burrowed upwards in an agar-filled tube when tested immediately, but downward when incubated in liquid medium for more than 30 min. Up or down burrowing was influenced by artificially inverting magnetic cues, suggesting that vertical migration in these conditions reflects an orientation to primarily magnetic cues rather than gravity cues. Likewise, this strain accumulated at a 300° from the northern direction of a uniform (earth strength) magnetic field applied across an assay plate if tested immediately, but 180° away (120°) if tested after 30 min in liquid medium. We found this preference for migrating at two angles 180° away from each other depending on time in liquid medium for all six conditions tested in our previous study (Vidal-Gadea et al. 2015). This included three independent behavioral assays and three different wild-type strains isolated from different global locations.

Other factors critical for migratory angle and overall magnetotaxis performance included the geometric layout for how the magnetic field enveloped the assay, as well as the humidity level in the lab. Here we test how temporal, spatial, and environmental factors contribute to migratory preference for the common wild-type lab strain N2. We find evidence that worms change their preferred orientation angle over the course of a 90-min assay with periods of no apparent preference. Understanding the preference of N2 strain worms for certain angles in a uniformly linear magnetic field of earth strength allowed us to predict how they accumulate in a radial magnetic field. These data concur with new results independently acquired by a lab new to assaying *C. elegans* behavior. With this additional information, we hope that more researchers will be drawn to study the cellular molecular basis for magnetoreception using *C. elegans*.

Materials and methods

Animals

C. elegans wild-type strain N2 was obtained from the CGC and raised on OP50 bacteria unless otherwise specified according to established methods (Brenner 1974).

Caldart and Golombek magnetic assay

A modified magnetotaxis assay developed by Caldart and Golombek was performed in Buenos Aires, Argentina. Nematodes were subjected to light:dark cycles (LD, 400:0 lux 12:12 h) under constant temperature (17.5 °C). Nematode populations were synchronized to the same developmental stage by the chlorine method (Lewis and Fleming 1995). The harvested eggs were cultured overnight in a 50-ml Erlenmeyer flask with 3.5 ml of M9 buffer (42 mM Na₂HPO₄, 22 mM KH₂PO₄, 85.5 mM NaCl, 1 mM MgSO₄) + antibiotic–antimycotic 1× (Gibco, Carlsbad, CA), at 110 rpm, 18.5 °C and under LD 12:12 h conditions.

The next day, L1 larvae were transferred to NGM (nematode growth medium) plates with *E. coli* strain HB101 to develop up to L4 stage. Then 500 individuals were transferred to NGM plates with *E. coli* HB101. For the magnetic migration assay, natural magnets (neodymium) were magnets positioned off to one side of the plate, providing an effective magnetic field of 700 Gauss (around 100 times the strength of that of the Earth) on the plate. To avoid any magnetic field, the control group was placed into a Faraday cage, and plates were covered with an iron mail with a cape of zinc, of hexagonal design. This mail was covered with aluminum foil for a better magnetic insulation. The experimental plates with the magnet were also covered with aluminum foil, with the magnet inside the package (without contacting the metal).

At the onset of the test, the nematodes were placed in the center of the plates, which were imaged 30 min later with a digital scanner (1800 dpi resolution). The agar plate was digitally divided into eight octants numbered in a clockwise fashion (with the magnet in the junction of octants one and eight). The number of worms was counted with the particle analyzer plug-in of Image-J software, and individuals present in each octant were assessed as the proportion of total worms in the plate. Migration was analyzed with a Rayleigh test to obtain the clustering as well as the significance of the resulting vector.

Two nematode strains were used in these assays: *C. elegans* strains N2 (Bristol strain as wild type), and PR671 *tax-2(p691)*, provided by the *Caenorhabditis* Genetics Center.

Preferred migratory direction over time

Worms were assayed for their preferred migratory direction as a function of time in Normal, IL, USA.

Animals

30–50 day 1 adult worms were used in each assay as previously described (here and Vidal-Gadea et al. 2015). Animals came from bleach-synchronized plates and were never starved, overpopulated, or infected. Each culture plate contributed to exactly one assay. Worms grew in a temperature- and humidity-controlled room. No experiments were conducted if environmental weather events caused humidity or temperature fluctuations above 5° or % humidity.

Assay plate

Animals were transferred using a 0.5- μ l droplet of liquid NGM (pH 7) in the center of a 1-day-old 10-cm chemotaxis assay plate. Sodium azide (0.1 M) was painted with a paintbrush on the circumference of the assay to paralyze animals at the edge of the plate following dispersal from the center, as described before (Vidal-Gadea et al. 2015). Animals were released to behave by carefully soaking the liquid NGM where they were trapped using a small piece of Kimwipe.

Magnetic coil system

We used a 1-m³ magnetic coil system consisting of three independently powered four-coil Merritt coil system as previously described (Vidal-Gadea et al. 2015) except for one important feature: our new system is double wrapped (Kirschvink 1992). Temperature and magnetic measurements were performed before and after each experiment to confirm our experimental conditions. A small fan circulated air through the volume of the magnetic coil system to prevent temperature gradients from building up.

Magnetic assays

We performed three types of assays. A unidirectional homogeneous magnetic field of 0.65 Gauss (one earth strength) was produced across the horizontal plate within the test volume (test condition, $N=7$). We ran magnetic controls in which the magnetic coil system was used to generate a magnetic field equal and opposite to that of the earth. This canceled-magnetic fields inside the test volume (magnetic control, $N=10$). Next, we powered our magnetic coil system to generate a 0.65-Gauss field once more but after attaining this field we switched the double-wrapped coil system into its anti-parallel configuration where the field generated canceled itself but produced the same power output as our test condition (current control, $N=6$). Before each assay, we rotated the magnetic coil system to a random starting position. To determine possible temperature gradients, we measured the temperature difference between the center of

the assay plates and its edge (5 cm) throughout the assays for each condition as previously reported in Vidal-Gadea et al. (2015).

Estimation of magnetic field

We used a DC milligauss meter model mgm magnetometer (Alphalab, Utah) to experimentally measure the magnetic field across the assay volume.

Filming

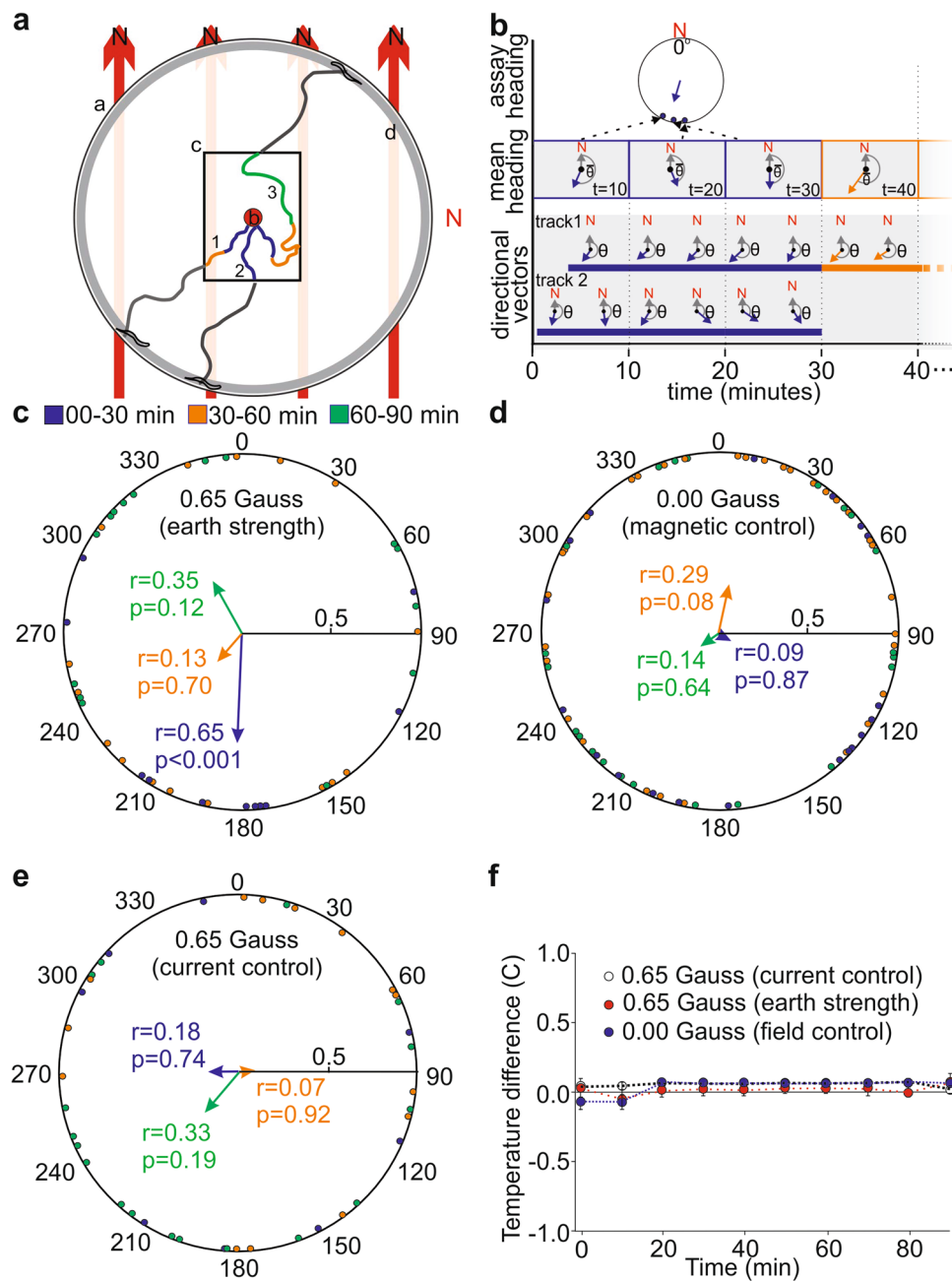
We used a USB camera (Plugable) driven by Micro-Manager software to film the magnetic assay. Two LED light sources were used to illuminate the filming arena. Test images were obtained and quantified using ImageJ to ensure no brightness gradients were present across the entire filming arena (measuring 37 \times 26 mm). Worms were filmed at 1 fps for 100 min and sampled at 0.2 Hz (every fifth frame) for heading analysis. Both USB camera and USB lights were wrapped in a grounded Faraday fabric made of copper. The same material was used to completely enclose the assay and prevent any electric fields from intruding in our assay.

Analysis

Worm centroids were tracked using ImagePro7 object tracking feature by experimenters blind to assay treatment. Animals had to move greater than 5 mm from the center starting position they were considered participants in the assay. The heading of each worm was obtained by custom-made script in Spike2 (Bainbridge et al. 2017). The script used animal centroid positions to determine headings over time. This was done by binning the track into equivalent intervals over which we sampled instantaneous directional vectors (Fig. 1). To normalize track segments, we binned animal trajectories into 5% segments to control for differential weighting of longer or shorter duration tracks. This method allowed us to sample the track in proportionally equivalent segments.

Directional vectors were determined by calculating the angle between the directions of locomotion in each 5% track interval relative to the direction of magnetic north. Each directional vector was given a time stamp that fell into ten windows of equal duration (10 min each). Each time window was assigned based on when a track segment began. To analyze temporal factors contributing to headings we took the mean of directional vectors across animals within a time window (e.g., 10 min, 20 min, etc.). This ensured that headings were weighted equally, and that each time window contributed one heading for an assay.

Because previous results indicated animals change directional preference in magnetic fields within 30 min from food, we grouped directional vectors into 30-min



time intervals (time windows 1 through 3, 4–6, 7–9) for a total of 90 min out of the 100-min assays. Mean headings within time intervals were pooled across assays to obtain population headings for each 30-min interval across assays. This ensured that each assay contributed one heading to the population heading for each 10-min window in a 30-min interval. Analyzing headings by 30 min intervals provided a conservative means to obtain population headings over each time interval. We used CircStat2012a module for Matlab to obtain the mean headings and circular plots for worms over the 30-min intervals described.

Six-point magnetotaxis assays

These assays were performed in Austin, TX, USA, and represent a modified version of our previously described magnetotaxis assay (Vidal-Gadea et al. 2015) with two main differences. First, droplets (1 μ l) of 1-M sodium azide were placed on six spots radially arranged around the starting position. Second, the magnet was moved so that it was beneath and adjacent to the plate, rather than directly beneath the upper quadrant of the plate. The magnet was also covered in a 0.5-cm plastic barrier to minimize the formation of a temperature gradient across the plate. First-day adult worms were

Fig. 1 Time dependence of magnetic orientation in *C. elegans*. We tracked the centroid of freely moving worms in our double-wrapped magnetic coil system. **a** Schematic of assay and recording field of view. We recorded freely behaving animals on assay plates (a) with a homogenous unidirectional magnetic field (red arrows) applied parallel to the agar surface. Animals were tracked once they passed a radius of 5 mm away from the center start position (b) until they left the 36×27 mm field of view (c). Animals could exit the center start position or the field of view at different times as exemplified by the numbered tracks (1–3) shown here in colored in blue (0–30 min), orange (30–60 min) and green (60–90 min). Once animals approached the edge of the assay plate, they were paralyzed by sodium azide (d). **b** To calculate animal headings over time we sampled animal headings by calculating directional vectors based on the angle of each track segment relative to magnetic field direction in 10-min windows (across 0–90 min). Directional vectors were grouped within each 10-min window (e.g., 0–10 min, 10–20 min, etc.) across tracks to determine a mean heading for that time window. Each 10-min window provided one mean heading for the assay. Mean headings were then placed in 30-min intervals (0–30-min, 30–60-min, 60–90-min intervals). Tracks 1–2 are colored here to reflect the time intervals from (a). Mean heading contributions for the 30-min interval are shown in the inset image. **c** Mean headings for animals in a 0.65-Gauss (1×earth) magnetic field. $T=0-30$ min: 183° , $r=0.65$, $p<0.001$; $T=30-60$ min: $r=0.13$, $p=0.7$; $T=60-90$ min: $r=0.35$, $p=0.12$. Length of vectors reflect proportion of headings clustered in a particular direction (e.g., radius=one means every heading was identical). Circles represent mean headings contributing to 30-, 60-, and 90-min intervals. **d** Mean headings for animals in 0.00 Gauss field (achieved by canceling out earth's field). $T=0-30$ min: 132.9° , $r=0.09$, $p=0.87$; $T=30-60$ min: 13.28° , $r=0.290$, $p=0.08$; $T=60-90$ min: 232.2° , $r=0.14$, $p=0.64$. **e** Powering the coil system under as in (a) but in their anti-parallel arrangement used similar currents to generate two anti-parallel magnetic fields that canceled each other out (current control). Under current control conditions $T=0-30$ min: 265.13° , $r=0.18$, $p=0.74$; $T=30-60$ min: 90.61° , $r=0.07$, $p=0.92$; $T=60-90$ min: 220.5° , $r=0.33$, $p=0.19$. **f** Temperature fluctuations within coil system over the course of our 90-min assays. Headings calculated using individual assay means (number of assays: 1×earth $N=7$, magnetic control $N=10$, current control $N=6$). Animal headings were tested using Circular Toolbox for Matlab. Rayleigh tests measuring circularity. Temperature measurements were obtained using high-sensitivity thermometers as previously described (Vidal-Gadea et al. 2015)

washed three times in NGM buffer, before being transferred to the center of the assay plate. Excess NGM from the puddle was wicked away, and worms were allowed to migrate for 30 min. At the end of the assay, worms paralyzed at each of the different points were tallied blind to position of the magnet. Significant deviation of the average portion of worms found at certain locations versus chance was assessed using a two-tailed comparison from mean (Zar 1999).

Humidity measurements

Humidity was measured using HC520 Digital Hygrometers. Hygrometers were allowed to equilibrate to ambient humidity both in and outside of the humidity chamber for 5 min before percent humidity was recorded to two significant figures. Measurements were taken daily for humidity in the

chamber, while ambient humidity was recorded at the beginning of each test performed outside of the chamber.

Statistics

Vectorial data were analyzed as previously described (Vidal-Gadea et al. 2015) using Circular Toolbox for Matlab (Mathworks). Following Landler et al. (2018), animals were not pooled but each assay mean heading was rather treated as a unit. We conducted Rayleigh tests to determine probability of deviation from a random von Mises circular distribution. Non-parametric groups were compared using Mann–Whitney ranked sum tests.

Results

Temporal factors influencing magnetic orientation

Previously, by studying how worms accumulate at different locations on a magnetic assay plate, we showed that populations of *C. elegans* migrate in different directions with respect to a uniform artificial magnetic field (Vidal-Gadea et al. 2015). These migratory directions appeared to correlate with various factors including the time elapsed between a worm being removed from its cultivation plate and the initiation of the migratory behavior on the assay plate. To directly measure how animals change their migratory angle over time, we filmed worms migrating in a homogeneously linear (earth strength 0.65 Gauss) magnetic field aligned parallel to the assay plate's surface. Using machine vision we extracted the centroid of each worms as it crawled freely away from the center of their assay plate (see “Materials and methods” and Fig. 1 a, b). All assays began within 5 min of worms being collected from their cultivation plate and lasted 90 min in total. Animals began at the center of a circular assay plate, and migrated freely until reaching the edge of the plate, where they became paralyzed by sodium azide to remove them from the assay.

For each assay, we tracked body centroids to obtain animal trajectories. Centroid-based trajectories have both spatial and temporal components, which we used to calculate headings and their change with respect to an imposed magnetic field over time. We observed that animals displayed variability regarding when they crawled away from the center of the plate (Fig. 1a, Sup Video 1). To track migratory preference over time, we broke the 90-min assay into nine 10-min intervals. Because we filmed animals over a 36×27-mm field of view over their start position, some animals crawled across this distance relatively quickly, while other animals remained in the field of view for longer times. To prevent animals from contributing unequally to the population heading calculation, we segmented animal trajectories

into 5% intervals. Each of these intervals was associated with one of the 10-min windows and was used to calculate the average heading for that animal over a 10-min period. This approach of normalizing tracks by periodic sampling of direction has been used to determine directionality in the case of *C. elegans* locomotion over long timescales (Peliti et al. 2013, Fig. 1b, and “Materials and methods”).

Animal headings were determined by taking directional vectors of animal locomotion during these 5% segments. We calculated directional vectors by taking the direction of animal locomotion within a 5% segment of the track relative to the applied magnetic field (0° being in the direction of the magnetic field). We calculated a mean heading for each 10-min window within an assay by averaging directional vectors across animals within each time window (Fig. 1b).

Because our previous observations indicated a change in magnetic orientation on a scale of 30 min, we grouped mean headings from 10-min windows into 30-min time intervals (0–30 min, 30–60 min, 60–90 min). We hypothesized that if worms change their migratory angle over time, then we should detect an increase in the variability of their migratory heading as time progressed. Furthermore, based on our previous findings, we further predicted that migratory preference for the population should tighten once again to a direction opposite their original preferred angle (Vidal-Gadea et al. 2015).

Figure 1 shows the results for the headings of the animals over the course of a 90-min assay. Over the initial 30 min, the mean heading of worms was 183.2° ($r=0.65$, $p<0.001$). Consistent with our prediction, between the 30- and 60-min mark the mean direction of the population became scattered (heading = 210.2°, $r=0.13$, $p=0.70$). Finally, beyond 60 min (60–90), worms displayed a non-significant trend toward the opposite direction (341° avg, $r=0.35$, $p=0.12$). It is worth noting that while this counter-migration is less robust than initial magnetotaxis of fed worms, animals that showed a reversal of migratory direction in our previous study were deliberately starved before release into the assay, whereas animals in this study were allowed to shift into a starved state while freely orienting in the assay. This difference could help account for differences in robustness observed over 90 min. These results are consistent with our previously reported findings of how worms accumulated at different angles after being tested immediately or 30-min after being collected from their cultivation plate (Fig. 2e, f in Vidal-Gadea et al. 2015).

Because electrical generation of magnetic fields in coils implies the unavoidable generation of heat, we used a fan to circulate air through our magnetic coil system and included two controls in our magnetic assays to control for magnetic field and temperature. We canceled out all magnetic fields inside our coil system to test worms in the absence of a magnetic field (magnetic control, Fig. 1c). In addition, we

controlled for heat by running the same current through the coil system in anti-parallel configuration (temperature control, Fig. 1d) thus generating a similar heat signature as that produced during our test. Importantly, we recorded and reported temperature changes in our system throughout each experiment. Worms assayed in canceled-magnetic field at 30 min (132.9°, $r=0.09$, $p=0.87$), 60 min (13.28°, $r=0.290$, $p=0.08$), and 90 min (232.2°, $r=0.14$, $p=0.64$) displayed no significant migratory preference throughout the 90-min duration of the assays. Likewise, our anti-parallel controls displayed no significant migratory preference at 30 min (265.13°, $r=0.18$, $p=0.74$), 60 min (90.61°, $r=0.07$, $p=0.92$), or 90 min (220.5°, $r=0.33$, $p=0.19$). Moreover, temperature differences measured inside our system across the 3.4 cm diameter of the assay plate were not significantly different from zero and maximally ± 0.1 °C during the 90-min period (Fig. 1d). Thus, at worst, without air circulation this may cause a radially symmetric gradient of ~ 0.03 °C/cm which is still $6\times$ less than the most shallow spatial gradient tested for *C. elegans* (0.20 °C/cm) in thermotaxis (Jurado et al. 2010). These additional controls support the idea that *C. elegans* changes the preferred angle of orientation to an earth strength magnetic field rather than to electrical or thermal cues that may emanate from our magnetic coil system.

Spatial factors influencing magnetic orientation

Given the observed preference for migrating at an angle in a uniform magnetic field, how do worms move in different-shaped magnetic fields? We previously tested how worms migrate from the center of an assay plate with a disc-shaped magnet placed under one side. With this arrangement, the magnetic field pierces throughout the plate directed radially outward from the center of the magnet (Fig. 3a–c in Vidal-Gadea et al. 2018). We had found that worms in this magnetotaxis assay accumulated on average more towards the magnet side than the control side (Vidal-Gadea et al. 2015).

To help explain this phenomenon, we recently proposed that N2 strain wild-type worms, which prefer orient $\sim 120^\circ$ to magnetic north, would be expected to migrate in a leftward arc towards the magnet if observing the assay from above (Fig. 3c in Vidal-Gadea et al. 2018). Indeed, this is exactly what we found when checking tracks left by worms in our previous 2015 study—most worms made leftward-arc tracks towards the magnet (Fig. 3f in Vidal-Gadea et al. 2018). Postdictions derived from theoretical analysis such as this one can be a powerful way to test and constrain novel understandings of biological mechanisms (Abbott 2008).

To test whether this theory could also predict new results, we conducted new magnetotaxis experiments with a modified setup. We dropped spots of azide to immobilize worms at six symmetrical points centered around the starting

position at the middle of the plate (spots 1–6, Fig. 2a). As before, the magnet was positioned beneath and adjacent to the plate with magnetic north-facing upward so that the horizontal component of the magnetic field pointed away from the magnet. A population of N2 worms was released at the center of the plate and allowed to crawl freely for 30 min. We tallied the number of immobilized worms for each of the six spots. As in our previous study, we predicted that most worms would migrate in a leftward arc towards the magnet with the a priori hypothesis that most worms would migrate to point 6 and fewer worms would migrate to point 3 (Fig. 2a, purple vectors). Indeed, we found that the majority of worms migrated leftwards towards the magnet towards point 6 (Fig. 2b). This was apparent because most worms in each assay were found at the upper left point 6 and far fewer worms were found at the lower right point 3. Both of these groups were significantly different from chance (point 6, $t=2.96$, $p<0.01$; point 3, $t=4.60$, $p<0.001$; $n=19$ assays, Fig. 2b). The results not only fit with our a priori hypothesis for the two points (3 and 6), but displayed an expected trend for the distribution of worms across the six points with a lower than chance level for points adjacent to 3 (2 and 4) and a chance level for points adjacent to 6 (1 and 5).

Replication of magnetic orientation behavior in *C. elegans*

Since the initial description of magnetic orientation behavior in *C. elegans* by the Pierce lab at University of Texas at Austin (Vidal-Gadea and Pierce-Shimomura 2012), several groups joined the study of magnetic field detection using nematodes. After our original 2012 study, Ilan et al. (2013) reported that parasitic nematodes migrated preferentially south over north when placed in a magnetic field. Additionally, the Golombek lab at the University of Quilmes, Argentina, replicated our findings with minor modifications. The Golombek results obtained in complete independence from the Vidal-Gadea and Pierce labs in 2016 are presented here.

To avoid potential shifts in orientation preference that depend on time in liquid media, assays were conducted in agar plates seeded with *E. coli* bacteria as food and for only 15 min. Second, to minimize potential temperature gradients introduced by magnet proximity, magnets were positioned off to one side of the plate and recorded the number of worms in each of eight wedge-shaped zones of the circular plate. Similar to Vidal-Gadea et al. (2015), we observed significantly more worms congregating near the north-side facing up magnet (Fig. 3a, Rayleigh clustering test, $p<0.05$, $n=12$) compared to worms tested in control conditions without a magnet (Rayleigh clustering test, $p=0.83$, $n=12$). Also consistent with our original study, we found that magnetic orientation did not achieve statistical significance for animals lacking a cGMP-dependent cation channel subunit

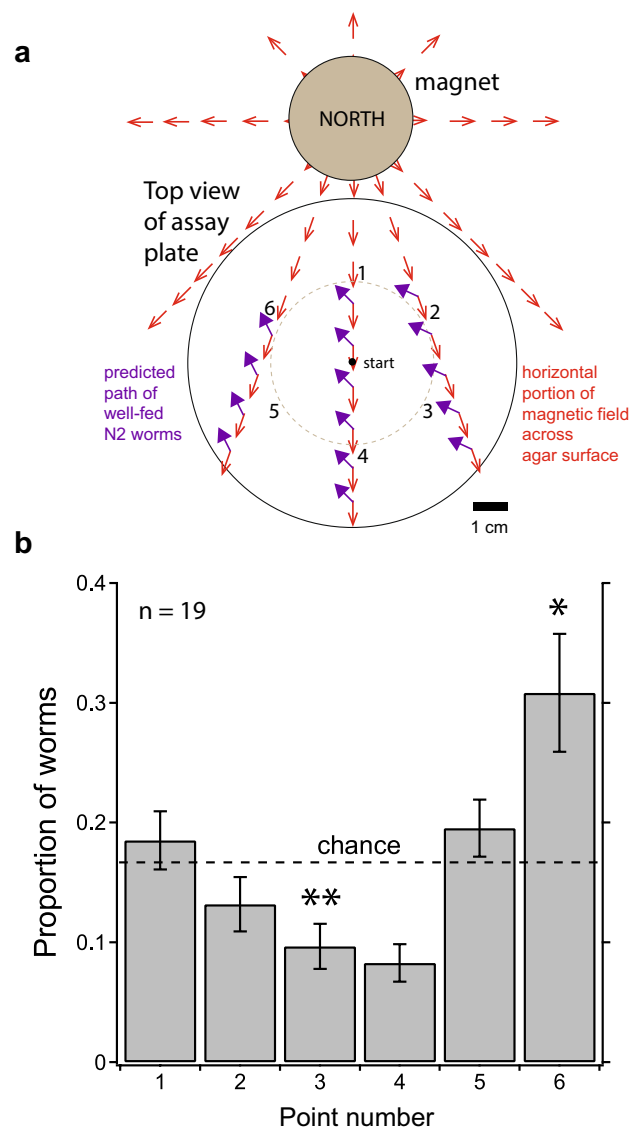
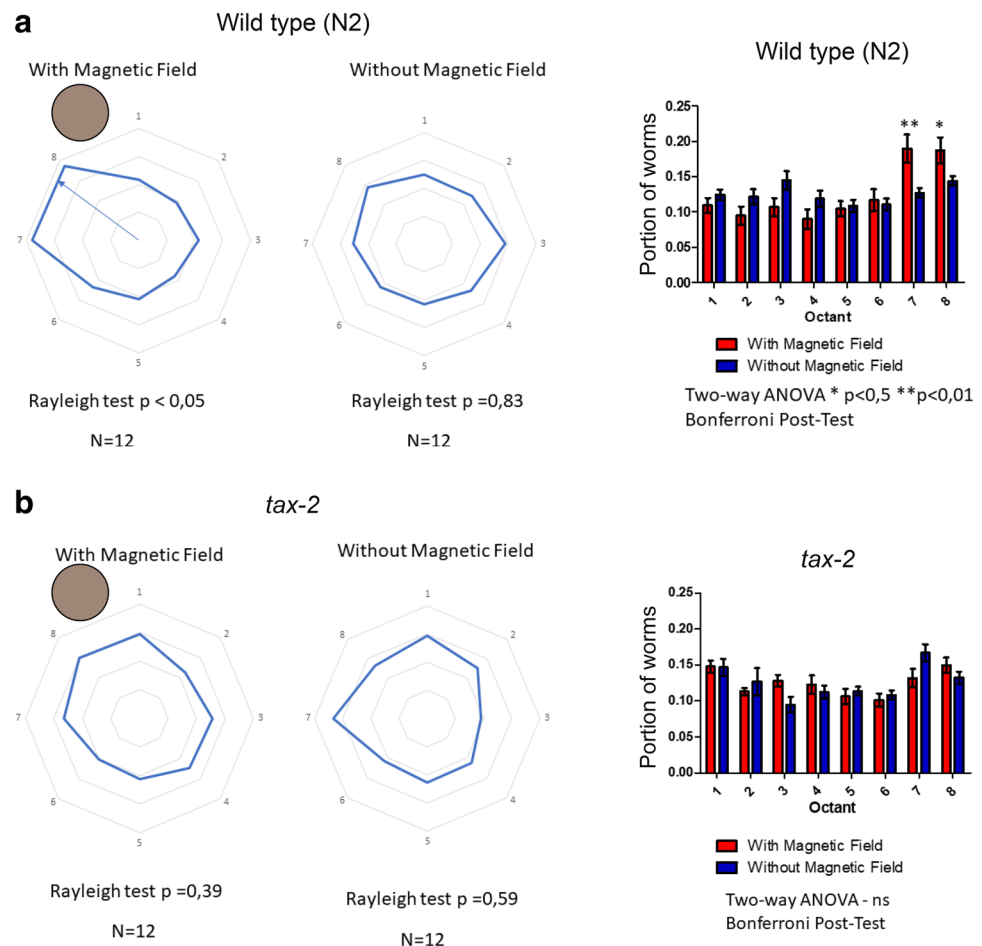


Fig. 2 Asymmetric accumulation in six-point magnetotaxis assay. **a** A group of worms was placed at center of the plate allowed to move freely. A north-facing magnet was positioned on one side beneath the plate. At the end of the 30-min assay, worms immobilized by azide at six points (1–6) were tallied with the experimenter blind to position of the magnet and identity of the points. Top view schematic displays horizontal portion of the magnetic field (red vectors, not to scale) across the surface of the agar-filled plate and predicted trajectory (purple vectors) of worms primarily towards point 6. **b** As predicted for a leftward-arc trajectory, compared to chance level of accumulation of 0.167, more worms accumulated at point 6 ($p<0.01$) and far fewer worms accumulated at opposite point 3 ($p<0.001$). Bars represent s.e.m. $N=19$ assays

tax-2 (Fig. 3b, $p=0.39$, $n=12$ in the presence of a magnet, $p=0.59$, $n=12$ when no magnet was present). Moreover, the finding that freely moving worms accumulated on the left side of a north-facing up magnet after 15 min (Fig. 3a) fits with our prediction and findings that worms migrate to the left side of a north-facing up magnet (Fig. 2).

Fig. 3 Independent evidence for magnetic orientation in *C. elegans*. Caldart and Golombek at the University of Quilmes, Argentina, performed a modified version of the magnetotaxis assay described in Vidal-Gadea et al. (2015). Well-fed worms were placed at the center of a plate seeded with bacterial lawn with no sodium azide. The fraction of worms found in each octant at 15 min is reported on the right. **a** Wild-type N2 worms spent more time in octant to the left of a strong neodymium magnet placed adjacent to the assay plate compared to paired assays where there was no magnet.

b By contrast, *tax-2* mutant worms showed no preference for any octant in magnet versus no magnet conditions. $N=12$ assays for each condition. Bars represent SEM



Environmental factors influencing magnetic orientation

Over the past 5 years of conducting magnetic orientation assays, we anecdotally noticed that *C. elegans* orients less robustly to magnetic fields on rainy days. We later learned that a similar relation for thermal orientation assays was recently reported for *C. elegans*. Leaders in the field of thermotaxis recommended excluding thermotaxis assays if humidity levels are above 50% relative humidity (RH) (Goodman et al. 2014). Because we had found that magnetic orientation also relies on the AFD neurons that are similarly pivotal for thermotaxis, we hypothesized that magnetic orientation performance may also show high variability above 50% RH. To test this idea, we reanalyzed data from our six-point magnetotaxis assay above (Fig. 2d, e) by replottting the average of assays conducted on dry or humid days separately with a threshold of 50% RH. We found that assays conducted on dry days exhibited more robust results than those conducted on humid days (Fig. 4a). Worms assayed on dry days showed a 93% higher average bias to accumulate at the left side of the magnet compared to worms assayed on humid days (point #6 values for orange dry avg line vs blue humid

avg line in Fig. 4). This reanalysis hints that humidity may lower robustness.

Discussion

Magnetic orientation is a fascinating behavior that many animals display in the wild but has proven more difficult to study in laboratory settings (Clites and Pierce 2017). Although animals are known to orient at certain directions with respect to the geomagnetic field in the wild, they are often found to orient at different directions when tested in the lab or in more controlled conditions (e.g., a recent example, Dreyer et al. 2018). This is usually interpreted to suggest that animals use additional sensory cues in tandem with magnetic field of the earth to guide their migrations. Others report difficulty replicating magnetic orientation studies (e.g., Landler et al. 2018).

Understanding factors that contribute to robust magnetic orientation in the commonly studied nematode *C. elegans* may enhance opportunities to study this poorly understood sensory modality and associated behaviors. We have found that timing and potential satiation state

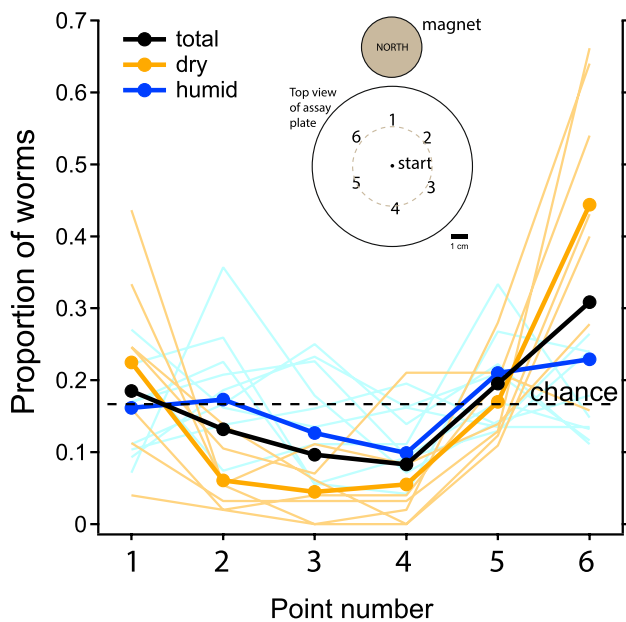


Fig. 4 Robustness of magnetic orientation behavior correlates with humidity. Results from six-point assay from Fig. 2d analyzed to investigate the potential effects of humidity on magnetic orientation performance. Average of trials of worms assayed on dry days (average 35.4% RH, $n=7$) shows more robust orientation towards left of the north-facing up magnet than worms assayed on humid days (> 50% RH, average 60.8% RH, $n=12$). Results from individual trials color-coded for dry or humid days (thin orange and blue lines, respectively) show consistent relation across trials

are two of the most controllable variables affecting magnetic orientation assays for *C. elegans*. In Vidal-Gadea et al. (2015), we noted that crowding, ambient humidity, temperature, starvation, and contamination history can all sway the preference of a population from positive to negative magnetotaxis (or indeed abolish the behavior altogether). This is perhaps not surprising given that the polymodal AFD neurons respond to temperature, humidity, and CO_2 in a satiation-dependent manner (Mori 1999; Bretscher et al. 2008; Russell et al. 2014). Leaders in the field of thermotaxis have similarly decided to try to avoid testing animals during weather events (e.g., rain, high humidity, temperature fluctuations) where relative humidity rises above 50% (Goodman et al. 2014). Although many behaviors in *C. elegans* are not noticeably affected by high humidity, other behaviors may be affected by high humidity (e.g., Dr. Cathy Rankin, personal communication). In our own labs, we have constructed an environmental box that maintains animals in constant temperature and humidity. Importantly, we have found that cultivating worms in an incubator (as was done in Landler et al. 2018) can interfere with the robustness of performance, perhaps due to strong magnetic fields cast by the incubator throughout development and/or potential shock after

worms are moved to a different temperature or magnetic conditions for testing. We feel it is crucial to know the state of the worms before and during assays.

We do know that once a permissive physiological state has been produced in the animals, they will perform this behavior. Two lines of evidence substantiate this. First, an independent group in Argentina recapitulated our results by conducting the experiments on a bacterial lawn and thus avoiding the risk of on-assay starvation. Their results are included in this study to provide important step forward in validating magnetic orientation in *C. elegans* with independent replication. Second, we regularly have undergraduate students, high-school volunteers, and even students with special needs replicate our results in Texas and in Illinois when handed worms properly cultured and assayed on dry days.

Alternatively, some animals fail to orient to magnetic fields at certain life stages when tested by researchers coincident with periods when they would not migrate in the wild. For *C. elegans*, we and others have found that although worms can orient to chemical, thermal, and humidity gradients from L1-stage larval through adulthood, larval-stage and old adult worms cannot perform magnetic orientation (Ward 1973; Bainbridge et al. 2016). Efficient magnetic orientation correlates with the presence of microvilli on the AFD sensory neuron that we previously found respond to magnetic fields and are necessary for this behavior. Transgenic worms lacking the glia required for the formation of microvilli on AFD neurons also fail to perform magnetic orientation (Vidal-Gadea et al. 2015).

Intriguingly, although thermotaxis is reportedly less robust in high humidity, the thermosensory ability of the AFD neuron appears to be the same whether the worm is assayed immersed in water or when allowed to crawl freely with air above, and even when the AFD neuron is cultured in liquid (Kimura et al. 2004; Kobayashi et al. 2016). Taking a cue from those who study thermotaxis in *C. elegans*, this suggests that magnetotaxis studies may progress on humid days by collecting more data to view significant results through more variable data, and/or by investigating the factors that affect magnetic orientation at the behavioral and the cellular levels.

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Compliance with ethical standards

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

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