

Do leaf-cutter ants *Atta colombica* obtain their magnetic sensors from soil?

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Abstract How animals sense, process, and use magnetic information remains elusive. In insects, magnetic particles are candidates for a magnetic sensor. Recent studies suggest that the ant *Pachycondyla marginata* incorporates iron-containing particles from soil. We used leaf-cutter ants *Atta colombica* to test whether soil contact is necessary for developing a functional magnetic compass. *A. colombica* is the only invertebrate known to calculate a path-integrated home vector using a magnetic compass. Here, we show that *A. colombica* requires contact with soil to incorporate magnetic particles that can be used as a magnetic compass; yet, we also show that ants can biosynthesize magnetic particles. Workers from a soil-free colony ignored a 90° shift in the horizontal component of the geomagnetic field, yet oriented homeward

despite the occlusion of any geocentric cues. In contrast, workers from a soil-exposed colony oriented to an intermediate direction between their true and subjective home in the shifted field. Homeward orientations under shifted fields suggest that ants calculated a path-integrated vector using proprioceptive information. Strikingly, ants from the soil-free colony also had magnetic particles; yet, as observed by ferromagnetic resonance, these particles differed from those in soil-exposed ants and were not associated with a magnetic compass sensitive to this experimental manipulation.

Keywords Homing · Navigation · Orientation · Path integration · Proprioception

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It is generally recognized that many animals use magnetic compasses to orient (Wiltschko and Wiltschko 1995; Johnsen and Lohmann 2008; Gould 2010; see also Winklhofer 2010 and review articles cited therein). Yet, the identification of a magnetic compass has largely remained elusive (Cadiou and McNaughton 2010; Kirschvink et al. 2010). In insects, eusocial Hymenoptera (especially bees and ants) have become target species for the study of the use, composition, and function of a magnetic compass (Wajnberg et al. 2010). Eusocial hymenopterans have been found to use a magnetic compass during foraging (Walker and Bitterman 1985; Banks and Srygley 2003; Riveros and Srygley 2008) and nest construction (Kisliuk and Ishay 1977; DeJong 1982; Vogt et al. 2008). Numerous behavioral assays as well as the presence of magnetic particles in different tissues suggest that a magnetite-based compass may be broadly extended in the group. So far, magnetic particles have been found in diverse body parts, including the abdomen, thorax, head, and antennae (Wajnberg et al. 2010 and references therein). Recent magnetometry and microscopy studies of the migratory ant *Pachycondyla marginata* Roger

further support the antennae as sites for the magnetic compass. Strikingly, magnetic particles in the antennae of *P. marginata* were suggested to be incorporated from soil (Oliveira et al. 2010), which challenges the current idea that the magnetic compasses comprise biomineralized particles (Kirschvink and Gould 1981; Alves et al. 2004; Abraçado et al. 2012).

Importantly, although the mechanism for incorporation of soil particles remains unknown, a testable behavioral consequence is that ants developing without soil should demonstrate an impaired magnetic compass. Aiming to test this idea, we investigated the effect of soil contact on the capacity of ants to rely on magnetic information. We investigate leaf-cutter ants *Atta colombica* Guérin-Méneville, which are major neotropical herbivores and nutrient recyclers (Herz et al. 2007; Wirth et al. 2003) and well-known for construction of long foraging trails to transport cut leaves to their nest (Wirth et al. 2003). Importantly, despite the marked use of pheromonal and physical trails for navigation, *A. colombica* uses a magnetic compass to determine directional information during path integration (Banks and Srygley 2003; Riveros and Srygley 2008). Foragers exposed to a strong magnetic pulse exhibit an impaired ability to determine a path-integrated vector home (Riveros and Srygley 2008), whereas the manipulation of magnetic polarity leads to a shift in vector direction (Banks and Srygley 2003; Riveros and Srygley 2008). Interestingly, when foraging nocturnally, only about 50 % of the ants in a colony respond to a shifted magnetic field (i.e., change their path-integrated vector as predicted), whereas strikingly, the other half orient towards true home despite manipulation of the magnetic field and the occlusion of other external cues. Proprioception is the perception of relative positions of neighboring body parts to measure movement without reference to external cues (Wehner et al. 1996). Hence, *A. colombica* is able to determine a path-integrated home vector by using a magnetic compass and/or a second mechanism, presumably based on proprioceptive information (Riveros and Srygley 2008).

Given these nest-oriented behaviors, it is possible to determine potential failures of the magnetic compass in *A. colombica* that are reflected in individual abilities to path integrate. Thus, we relied on foragers of *A. colombica* to evaluate use of magnetic information during path integration by individuals that developed in colonies isolated from soil and compared their performance with ants developing in natural conditions underground. Moreover, we searched for magnetic particles in ants from both soil-exposed and soil-free colonies and compared their physical properties. Based on our previous results in *A. colombica* and the findings from *P. marginata*, we predicted that ants from the soil-exposed colony should rely on a magnetic compass plus a proprioceptive compass, whereas ants from the soil-free colony should rely only on the proprioceptive information if contact with soil is required to develop a functional magnetic compass. As a result, (1) in under control (natural magnetic field) conditions, ants from

both colonies would be able to calculate a path-integrated home vector and (2) with the horizontal component of the local magnetic field shifted 90°, ants from the soil-exposed colony should exhibit an intermediate vector with some following their magnetic compass and others their proprioceptive compass. In contrast, ants from the soil-free colony should be insensitive to the magnetic shift and orient with their proprioceptive compass towards true home. We also expected that ants from the soil-free colony would lack ferromagnetic particles, whereas those from the soil-exposed colony would possess them.

Methods

Experimental animals and study site

We conducted our study in Gamboa, Republic of Panama (elevation 30 m; 9° 07'N, 79°42'W) during January 2010 at the beginning of the dry season. A mature colony of *A. colombica* naturally nesting outdoors in native soil was identified, and ant foragers were diverted from their trail toward our coil by attracting them with a trail of barley flakes. After 2 days of baiting, the ants built a new trail toward our feeder and maintained ca. 2 m from the coil (Fig. 1). The soil-free colony was collected in March 2009 and reared without soil or sand in transparent food storage boxes for 10 months in a laboratory (after Herz et al. 2008). Simulating underground chambers of a natural colony, seven 7×7 cm boxes containing the colony and fungal gardens were evenly spaced along a 0.7 m long tube (2 cm diameter). Simulating a foraging trail, the tube continued 0.5 m from the nest chambers without further interruption to another box into which hibiscus leaves *Hibiscus rosa-sinensis* L. cut daily from plants cultivated in Gamboa gardens were introduced and from which the colony had access to water. One characteristic of *A. colombica* is that the ants dispose of waste in a pile above ground, and so the tube continued ca. 0.5 m in the opposite direction from the colony to a small box into which the ants dumped refuse. Fluorescent ceiling lights were turned on during the day and off at night at irregular hours. For the experiment, we emptied the feeder box of leaves and connected a 15.9 m long plastic tube (5 cm diameter) from the feeder box in the laboratory to a second feeder that was placed on the ground outside of the lab building and ca. 2 m from the coil (Fig. 1). Moving from the nest to the feeder in the tubing, the ants passed through one complete concrete block wall in which there was only a hole big enough for the tube to pass, blocking both visual and olfactory cues from the nest, and a second three-quarter concrete block wall, which was screened at the very top.

Experimental setup

Ants' orientations were evaluated inside of a Rubens coil (1 m×1 m×1 m; Rubens 1945) oriented with its open faces

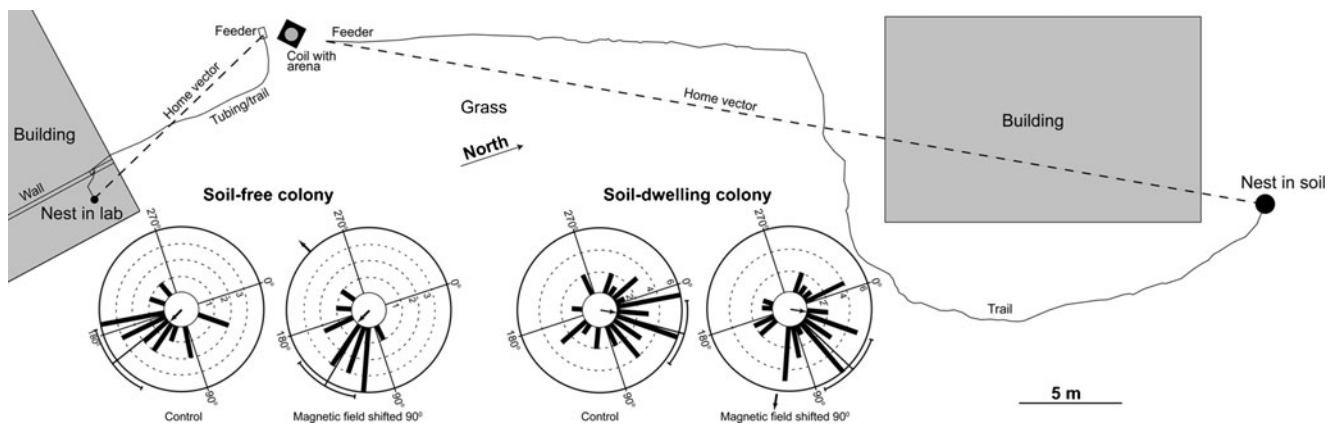


Fig. 1 Experimental setup including traces of the ant trails from the soil-exposed and soil-free lab colonies, predicted path-integrated orientations and orientations of individual ants in the different treatments. Vertical displacement in the trail of the soil-free colony is not shown and was not used in calculating the vector to the nest. Vertical displacement of the trail from the soil-exposed colony was negligible. Orientations of ants from each

treatment are plotted on circular histograms with the radius indicating the mean vector and the arc outside the circle representing its 95 % confidence interval. The *arrow* inside the circle is oriented towards the true home direction. For the experimental treatments, the *arrow* outside the circle is oriented toward the experimentally shifted home direction. *Values* inside the circles are the numbers of individuals in each treatment

to the southeast and the northwest. Measured locally with a magnetometer (Applied Physics System 3-axis model 113), the geomagnetic field intensity was 0.360 G with a horizontal component of 0.302 G oriented to geographic North (-3° declination, <http://www.ngdc.noaa.gov/geomagmodels/struts/calcDeclination>) and a vertical component of 0.195 G. For the experimental ants, 1 V was applied from an Extech DC power supply and a current of 1.38 A flowed through the coil yielding a total flux for the manipulated field of 0.360 G shifted 90° clockwise relative to true north. In addition to working on moonless nights (2045–0200 h), we enshrouded the coil with an opaque canvas tarp and created a black plastic door on one side to introduce individual ants onto the arena. The arena was a round plastic aquarium lid (internal and external diameters=60 and 61.5 cm). The study area is generally planar, and we also level led the arena at the center of the coil. The arena was filled with sand, which was mixed after each individual test to avoid the effect of chemical trails on orientation of subsequent ants. We were particularly cautious to run experiments when laboratory air conditioners were not operating to prevent the use of any seismic vibrations from these sources. Each ant's path was video recorded with a SONY TRW-700 camcorder taping at 30 frames/s. The camera rested on an opaque plastic table 80 cm above the arena with the lens projecting through the hole in the center of the table, which it filled, and the center coil. Above the arena, we centered a small two-LED reading light that evenly illuminated the arena and served to better visualize each ant's path with the camcorder. After all paths had been recorded, an observer blind to the treatments measured the vector defined by each ant's starting point near the center of the arena and the point where the ant first reached a line drawn on the monitor.

This line was located at least 2 cm within the arena's edge to avoid edge effects. Vectors were converted to orientation angles relative to geomagnetic north.

Experimental manipulations and analysis

We conducted our study at night, between 2045 and 0200 h during new moon. To transport an individual, we illuminated the feeder with a flashlight and gripped the barley flake that each ant held once it had started on its way back to the trail. With the flake, the ant was carried to the center of the arena and released onto the sand. Both colonies were unfamiliar with the sand substrate in the arena, and ants from both colonies were transported the same distance to the arena. Thus, ants from both colonies should have been stressed to the same degree prior to measuring their behaviors. Ants were tested in one of two conditions. In the control situation, the coil was off and the ants could use the naturally occurring magnetic field. Alternatively, experimental ants were evaluated under a magnetic field shifted 90° clockwise in its horizontal component. Every night, treatments were alternated starting with a control. Ants from the soil-free colony were tested on the first and last nights and those from the soil-exposed colony on the nights in between.

Ferromagnetic resonance spectroscopy

Following testing in the shifted field, ants were stored in 75 % ethanol and used in ferromagnetic resonance spectroscopy (FMR) measurements ca. 1 month after sampling. During preparation, all ants were carefully treated to exclude any potential contamination (most relevant in the case of ants from the soil-exposed colony). First, all ants were visually oriented in the angle 0 (as defined below) and the spectra were obtained with a Bruker

ESP 300E spectrometer (16 mW microwave power, 2.0 G field modulation, 8 scans, and 6.3×10^4 receiver gain). These acquisition parameters were used for all spectra except where stated. The absorption area (S) of these spectra, which is proportional to the magnetic material amount, was calculated as the second integral of the derivative spectrum starting at high field values where the baseline is better defined. To calculate S, custom software was developed using the graphic language LabVIEW. Second, the angular dependence of the spectra of 13 soil-free and 14 soil-exposed ants was measured with each ant's long body axis positioned in the vertical of the laboratory perpendicular to the spectrometer's magnetic field. Angle 0 was taken for the magnetic field parallel to the ant frontal plane. The ant was then rotated around its long body axis. Spectra of 3.8 mg of powdered local soil were obtained at three arbitrary orientations 45° apart from each other, rotating the sample tube around the vertical (receiver gain 6.3×10^2).

Statistical analyses

Orientation data were evaluated and plotted using the program Oriana (version 2.02, Kobach Computer Services). Analyses of magnetic spectral areas and ant mass were performed with INSTAT software. Tests for equality of variances and normal distributions of log-transformed S and mass were completed with JMP software (version 8.0, SAS Inc.).

Results and discussion

Path integration towards home

As expected from previous experiments (Banks and Srygley 2003; Riveros and Srygley 2008), ants from the soil-exposed colony evaluated under control conditions in the natural magnetic field oriented along a path-integrated vector towards their nest (Fig. 1, Table 1). Control ants from the soil-free colony also oriented homeward. These results are very robust because ants from the two feeder columns were displaced to the same arena for testing, and their respective nests were 126° apart. Furthermore, under our experimental conditions, all possible visual, chemical, and seismic cues were occluded (see experimental procedures). That ants from both colony would be able to orient homeward was expected since *A. colombica* workers may rely on both magnetic and proprioceptive compass (Banks and Srygley 2003; Riveros and Srygley 2008).

Responses to the magnetic shift

Ants from the soil-free colony appeared to be insensitive to the shifted magnetic field. The orientation of the ants in the shifted field did not differ from that of the controls (Table 1). Lack of

response to the magnetic field is evidence that the soil-free ants did not update their path-integrated vector with a magnetic compass sensitive to the horizontal component. Hence, results of both the control and experimental soil-free ants indicate that the nest location was reached from proprioception of turns in the foraging column and of rotation through the air when they were displaced from the foraging trail. The lack of response to the magnetic shift in soil-free ants is evident in the unimodal distribution of workers in a path-integrated direction towards true home (Fig. 1). This capacity to calculate a path-integrated vector based on egocentric information in ants further explains our results testing the ants from the soil-exposed colony.

When evaluated in the shifted magnetic field, ants from the soil-exposed colony significantly oriented in a direction (Table 1) that was between the true home (27°) and the subjective homeward direction (117°) under the shifted magnetic field (both the true and subjective homeward directions lay outside the 95 % confidence interval around the mean; Fig. 1 and Table 1). This reflects a conflict between a path-integrated home vector based on magnetic information (directed 90° clockwise from true home) and one based on egocentric information (directed towards true home). A similar conflict was evident in the orientation behavior of another soil-exposed colony assayed at night (Riveros and Srygley 2008). In hamsters, a compromise orientation is adopted when there is a conflict between the path-integrated home vector and a landmark cue (Ettienne et al. 1990). The soil-exposed ants tested also oriented in a direction intermediate to that indicated by the magnetic and egocentric cues.

Egocentric information is the use of proprioception rather than external cues to navigate. It involves integrating all angles steered and all distances covered into a home vector (Wehner et al. 1996). In combination with a skylight compass, egocentric cues are known to play an important role during path integration by ants (Wolf 2010), especially in determination of distance (Wittlinger et al. 2006) and inclination (Wohlgemuth et al. 2001). However, egocentric information as a directional compass has not been previously proposed in species other than *A. colombica*: when evaluated under a 180° shift in the magnetic field and in absence of any geocentric cues, workers of *A. colombica* oriented bimodally, either following the magnetic shift or orienting toward true home (Riveros and Srygley 2008). In vertebrates, turns occurring during navigation can be measured using proprioception, and thus, egocentric cues can be used to compute angles during path integration (Ettienne et al. 1996). In our case, individuals must compute angles while walking on the natural foraging trail or inside the tube and also compensate for rotation when transported through the air from the trail to the arena. Thus, our results suggest that workers of the leaf-cutter ant *A. colombica* are able to calculate a path-integrated vector based on egocentric cues as well as magnetic cues. Since ants are known to determine walking distance also from egocentric information (Wittlinger et al. 2006), our results

Table 1 Predicted and observed orientations of the ants from each colony

Comparison	Soil-free colony		Soil-exposed colony	
	Control	Field shifted	Control	Field shifted
Home vector length (m)		8.9		46.5
True homeward direction		153°		27°
Observed ($\mu \pm 95\%$ C.L.)	160 \pm 34°	139 \pm 24°	34 \pm 30°	62 \pm 32°
Uniformity (Rayleigh's test)	Z=7.84 N=18 r=0.66 P=0.0002	Z=9.60 N=15 r=0.80 P<0.0001	Z=11.24 N=40 r=0.53 P<0.0001	Z=10.40 N=40 r=0.51 P<0.0001
Observed control vs. observed shifted (Watson-Williams <i>F</i> -test)		F=1.53 N=18, 15 P=0.225		F=3.19 N=39, 40 P=0.078

point to the possibility that *A. colombica* is able to use path integration relying entirely on egocentric information. This intriguing possibility also leads to the question of the necessity of *A. colombica* to rely on path integration. Workers of the genus *Atta* are well-known for building foraging trails, thus, largely relying on pheromone and/or physical trails, but chemical cues alone do not provide polar information to the ants (Wetterer et al. 1992). A system to path integrate may be useful for scouts searching for new resources or in times when foraging trails are unavailable (e.g., after strong rains). A capacity to path integrate using magnetic or egocentric information might also be useful in more basal species of fungus growing ants (Myrmicinae: Attini) in which solitary foraging is the norm and nocturnal foraging is also common. A hierarchy of celestial over magnetic compass information is evident (Banks and Srygley 2003), and so magnetic information may be particularly useful in areas where celestial information is not available or when moving underground or foraging at night.

Ferromagnetic resonance spectroscopy

Our results following the experimental manipulation of the magnetic field exclude the possibility that ants from the soil-free colony could rely on the horizontal component of the geomagnetic field and demonstrate that ants acquire their magnetic compass from soil. Representative spectra of soil-exposed ants and of the local soil are shown in Fig. S1. These ant spectra contain an incomplete component at low field (LF), at $g > 4.3$, and a complex superposition of lines in the highfield (HF) region with g_{eff} from 1.9 to 3.9, arising from different magnetic particle systems. Both the LF (indicated by arrow) and HF (low intensity features in the circled region) are observed in the soil spectra (Fig. S1). Nevertheless, the soil spectra are dominated by a HF broad line (~ 750 Oe) at $g = 2.7$ not present in any spectra of the soil-exposed ants and also not observed in other soils, as far as we know. Note that the HF features in the soil spectra show an angular dependence. The

incomplete LF component is weakly noted as a base line shift. The dissimilarity between the soil spectra and the spectra of the soil-exposed ants indicates that the soil-exposed ants were selective in their incorporation of magnetic nanoparticles from the tropical soil, which is typically composed of a broad range of magnetic minerals that vary in size.

A lack of sensitivity of soil-free ants to the magnetic field shift does not preclude the possibility that soil-free ants also have ferromagnetic particles. Fig. S2 shows the spectra of all soil-free ants. The incomplete LF and the HF components are present. The HF component is clearly observed in Fig. S2a in the g_{eff} region from 2.3 to 2.6, and the LF is easily observed in Fig. S2b with the HF component falling across a wider range from 2.1 to 3.7. Qualitatively, the HF field component is more prominent than the LF component relative to spectra of soil-exposed ants.

Previous studies have shown the LF and HF components as characteristic of spectra of body parts of other social insects (Wajnberg et al. 2010 and references therein). The HF was associated with isolated nanoparticles while the LF was associated with large or aggregate particles. As soil-free ants cannot incorporate particles from soil, they must be biomineralized and should have controlled size, shape, and arrangement. As a result, they should also have similar spectra. The differences among the spectra (Fig. S2) arise in part from the differences in ant orientations as the ants cannot be positioned identically on the cylindrical wall of the quartz tube. Also, the antennae and body segments cannot be aligned precisely with the vertical. Differences in the ants' ages and functions can also influence the magnetic material amount and orientation as reported for honeybees (Gould et al. 1978). A more detailed analysis of the complexity of these FMR spectra is in progress.

FMR analysis shows that ants from both colonies had magnetic particles but that their properties differ. The area (S) of the FMR spectrum of each ant is proportional to the amount of ferromagnetic material. We found that the distribution of these relative quantities differed between the two colonies (Fig. 2).

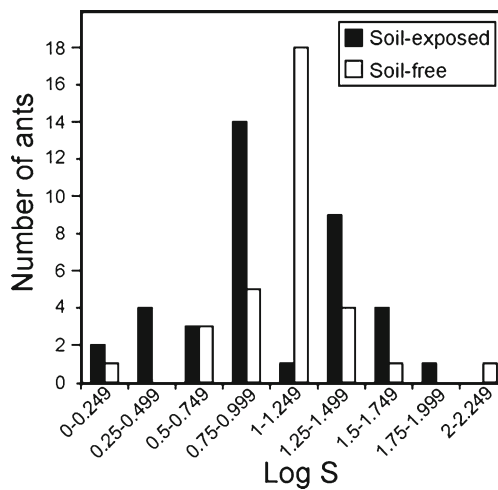


Fig. 2 Histograms of log-transformed spectral areas (log S) for soil-free and soil-exposed ants. S is proportional to the amount of magnetic material

Relative to the soil-exposed ants, soil-free ants were significantly more homogeneous in the amount of magnetic material in each ant (log-transformed S: mean \pm SD=1.06 \pm 0.33 for soil-free ants; mean \pm SD=0.98 \pm 0.44 for soil-exposed ants; Levene test for equal variances: $F_{1,69}=6.0$, $P=0.017$). In addition, the amounts of magnetic material for the soil-free ants were not normally distributed ($P=0.003$), whereas that for the soil-exposed ants followed a normal distribution ($P=0.34$). Moreover, this difference in the distributions of the areas cannot be attributed to body mass because soil-free ants (log-transformed mass, mean \pm SD=0.53 \pm 0.16 mg) were smaller than the soil-exposed ones (0.76 \pm 0.11 mg) and variances were similar (Levene test: $F_{1,72}=1.4$, $P=0.23$). The mean magnetic amounts are similar in ants of both colonies, but sensitivity to a magnetic sense is not necessarily associated with the amount of magnetic material. Instead, size, magnetization, and arrangement of the particles are all important parameters for magnetic field sensitivity based on biological magnetic-torque transducers of magnetite (Winklhofer and Kirschvink 2010). Moreover, the antennae of *P. marginata* ants have ferromagnetic nanoparticles that despite their small size and low magnetic susceptibility (e.g., hematite, goethite, ferrihydrite, and others) are theoretically capable of sensing the magnetic field torque due to their arrangement in a ferromembranous ellipsoidal cell (Oliveira et al. 2010). The relative heterogeneity in amount of magnetic material among the soil-exposed ants could arise from differences among the ants in obtaining ferromagnetic particles from the soil or from the soil-exposed ants having both soil-derived and biomineralized ferromagnetic particles.

We classified the angular dependence of FMR spectra of 13 soil-free and 14 soil-exposed ants into three groups (see Fig. 3 legend for characteristics of each): anisotropic in intensity (Fig. 3a), anisotropic in line position (Fig. 3b), and a third group that was isotropic (Fig. 3c). Soil-free ants were more likely to be

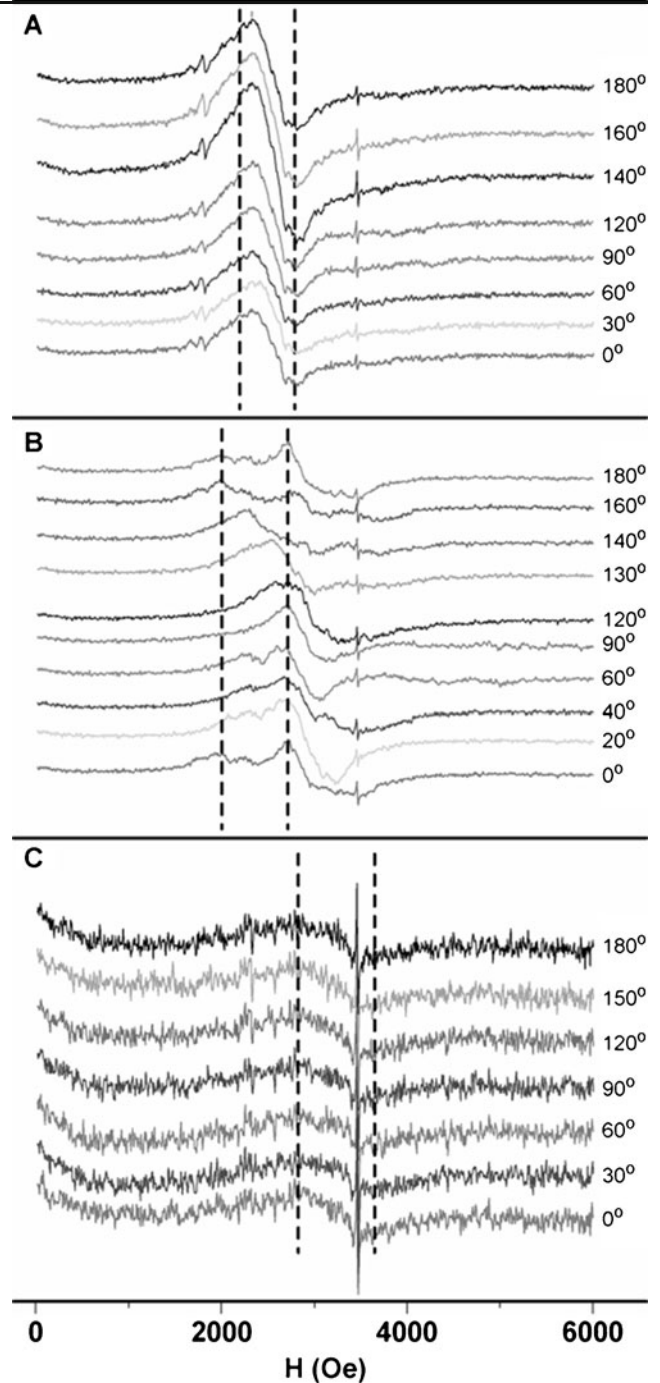


Fig. 3 Three types of FMR spectra from whole ants with the angle that each ant is rotated around its long body axis shown at right. In **a**, an ant from the soil-free colony shows anisotropy in intensity because the peak positions (indicated by the vertical dashed lines) are independent of the rotation angle, whereas the difference in peak intensities varies (140° being the highest and 30° the lowest for this ant). In **b**, an ant from the soil-exposed colony shows anisotropy in line position. Vertical dashed lines indicate peaks of two spectral components: one at low field and the other at high field values. For this ant, the low field peak varies from 2,000 Oe at 0° to 2,700 Oe at 90° when the spectrum appears as only one component at a high field value. In **c**, a soil-exposed ant shows isotropy because peak positions (indicated as vertical lines as in **a**) and peak intensities are independent of the rotation angle

anisotropic in intensity, and soil-exposed ones were more likely to be anisotropic in position ($X^2=8.06$, $df=1$, $P=0.004$). We tested whether ants collected from a second soil-exposed colony were more likely to be anisotropic in position. Consistently, we found that of seven ants that were collected from the *A. colombica* colony in Gamboa that was used in an experiment to disrupt the magnetic compass (Riveros and Srygley 2008), five were anisotropic in position, none were anisotropic in intensity, and two were isotropic. Anisotropic differences can be due to the material arrangement or to its magnetic structure. FMR results show that leaf-cutter ants *A. colombica* are able to biomineralize ferromagnetic material that differs in its properties from the particles collected from soil. Yet, ants do not respond to a shift in the horizontal component of the geomagnetic field unless they have access to the soil and are able to incorporate such particles in areas associated with magnetoreception.

In many eusocial insects, magnetic techniques and transmission electron microscopy point to the head and/or antennae as likely locations of magnetoreceptors (Wajnberg et al. 2010). In the migratory ant *P. marginata*, different kinds of iron-containing magnetic particles are found in the three most proximal joints of the antennae: between head and scape, scape and pedicel, and pedicel and the third antennal segment. A magnetoreception function is suggested by the presence of magnetic particles within tissues that are near to mechanosensitive structures and located in these very specific areas. In the pedicel-scape joint, a cell-like structure containing maghemite particles is assumed to be a proprioceptor because of its location near sensilla receptors. Nevertheless, other iron oxide particles can also be present, and theoretically, the structure would still be sensitive enough for the detection of the geomagnetic field even with weak magnetic materials such as hematite (Oliveira et al. 2010). Goethite and/or hematite particles were observed in the cuticular knobs in the joint between the pedicel and the third antennal segments. These particles might serve as statoliths for gravireception because the knobs are connected to the Johnston's organ, which among other functions senses gravity and can be involved in the temporary magnetic disorientation in ants (Vowles 1954).

In Gamboa, as in other regions of the tropics, the soils are reddish because they are rich in iron oxides, typically goethite and hematite. Plants take up Fe+2 (ferrous ions) for use in photosynthesis, respiration, and nitrogen and sulfur assimilation (Jeong and Guerinet 2009; Briat et al. 2010), and the leaf-cutter ants cut and carry leaves to the nest. Within the leaf-cutter ant nests are the fungal gardens *Leucagaricus gongylophorus*, which the ants tend and feed the leaf cuttings. The fungus is the only food for the ant larvae and practically the only food for the adults. In addition, there are many bacteria several of which are symbiotic to the fungus and the ants. Because many fungi and bacteria have siderophores specifically to actively transport ferrous ions into the cell,

these may be sources of iron to biomineralize the ferromagnetic material. Although it is not known whether *L. gongylophorus* has magnetite, some fungi, including *Fusarium* and *Verticillium*, are able to synthesize magnetite from ferric and ferrous salt mixtures (Bharde et al. 2006). Both soil-free and soil-exposed ants had access to water, plant leaves, and *L. gongylophorus* as possible sources of ferrous ions. In addition, *Streptomyces* bacteria, a species of which leaf-cutter ants collect from soil to protect their fungal gardens from invasive fungi (Seipke et al. 2011), secrete the siderophore desferrioxamine to take up Fe+3 and convert it to Fe+2 intracellularly. Soil-free ants would have lacked contact with soil-borne *Streptomyces*, and thus, these bacteria are good candidates for providing ferromagnetic material only to soil-exposed leaf-cutter ants. Biomineralization of magnetic nanoparticles by *A. colombica* is consistent with the process by which other eusocial insects that do not consume fungus obtain their magnetic particles (Wajnberg et al. 2010).

Altogether, the differences in response to a shifted magnetic field, the distributions of the amounts of magnetic material and anisotropy of magnetic particles suggest a new hypothesis to explain the differences between soil-free and soil-exposed colonies. We hypothesize that *A. colombica* ants from the soil-free colony for which magnetic particles can only be biomineralized appear to rely only on a proprioceptive compass or compasses to measure turns on the ground and rotations in the air to calculate with measures of distance the path-integrated vector to the nest independent of the horizontal magnetic information. For a proprioceptive compass, dense minerals are important and all of the iron minerals typically found in tropical soils are relatively similar in density. Thus, incorporation of iron minerals directly from soil into the proprioceptive compass would achieve similar results to biomineralization of particles. However, our results with ants from the soil-free colony clearly suggest that the magnetic particles, if truly involved in the proprioceptive system, are biomineralized. Natural, soil-exposed ants probably possess two magnetic particle types making their FMR signal more complex and with anisotropy in line position: biomineralized particles, as those of soil-free ants, and the soil-derived particles involved with a magnetic compass that is used to update their path-integrated vector homeward.

Conclusions

We conclude that leaf-cutter ants *A. colombica* require contact with soil in order to incorporate magnetic particles that can be used as a magnetic polarity compass. Absence of soil-originated particles impairs the ability to use a magnetic compass to update a path-integrated vector. However, this impairment is not associated with absence of magnetic particles. On the contrary, we suggest that *A. colombica* is capable

of biomineralization but the produced particles differ from the soil-derived particles in composition or arrangement and appear not to be related to this magnetic compass. The function of the biomineralized particles is not known but might be involved in a rotational sense like statoliths. If this were the case, *A. colombica* workers would naturally possess two compasses to calculate a path-integrated vector home: a magnetic compass derived from soil and a proprioceptive one derived from biomineralized magnetic particles. This hypothesis, which warrants further investigation, points to the possibility that *A. colombica* not only possesses a remarkable capacity to path integrate using a magnetic compass but also that the workers are able to calculate distance and direction relying on egocentric information.

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References

- Abraçado LG, Esquivel DMS, Wajnberg E (2012) ZFC/FC of oriented magnetic material in *Solenopsis interrupta* head with antennae: characterization by FMR and SQUID. *J Biol Phys* 38:607–621
- Alves O, Wajnberg E, de Oliveira JF, Esquivel DMS (2004) Magnetic material arrangement in oriented termites: a magnetic resonance study. *J Magn Res* 168:246–251
- Banks AN, Srygley RB (2003) Orientation by magnetic field in leaf-cutter ants, *Atta colombica* (Hymenoptera: Formicidae). *Ethol* 109: 835–846
- Bharde A, Rautaray D, Bansal V, Ahmad A, Sarkar I, Yusuf SM, Sanyal M, Sastry M (2006) Extracellular biosynthesis of magnetite using fungi. *Small* 2:135–141
- Briat JF, Duc C, Ravet K, Gaymard F (2010) Ferritins and iron storage in plants. *Biochem Biophys Acta* 1800:806–814
- Cadiou H, McNaughton PA (2010) Avian magnetite-based magnetoreception: a physiologist's perspective. *J Roy Soc Interface* 7:S193–S205
- DeJong D (1982) Orientations of comb-building by honey bees. *J Comp Physiol* 147:495–501
- Etienne AS, Teroni E, Hurni C, Portenier V (1990) The effect of a single light cue on homing behaviour of the golden hamster. *Anim Behav* 39:17–41
- Etienne AS, Maurer R, Seguinot V (1996) Path integration in mammals and its interaction with visual landmarks. *J Exp Biol* 199:201–209
- Gould JL (2010) Magnetoreception. *Curr Biol* 20:R431–R435
- Gould JL, Kirschvink JL, Deffeyes KS (1978) Bees have magnetic remanence. *Science* 201:1026–1028
- Herz H, Beyschlag W, Hölldobler B (2007) Herbivory rate of leaf-cutting ants in a tropical moist forest in Panama at the population and ecosystem scales. *Biotropica* 39:482–488
- Herz H, Hölldobler B, Roces F (2008) Delayed rejection in a leaf-cutting ant after foraging on plants unsuitable for the symbiotic fungus. *Behav Ecol* 19:575–582
- Jeong J, Guerinot ML (2009) Homing in on iron homeostasis in plants. *Trends Plant Sci* 14:280–285
- Johnsen S, Lohmann KJ (2008) Magnetoreception in animals. *Phys Today* 61:29–35
- Kirschvink JL, Gould JL (1981) Biogenic magnetite as a basis for magnetic field detection in animals. *Biosystems* 13:181–201
- Kirschvink JL, Winklhofer M, Walker MM (2010) Biophysics of magnetic orientation: strengthening the interface between theory and experimental design. *J Roy Soc Interface* 7:S179–S191
- Kisliuk M, Ishay J (1977) Influence of an additional magnetic field on hornet nest architecture. *Cell Mol Life Sci* 33:885–887
- Oliveira JF, Wajnberg E, Esquivel DMS, Weinkauff S, Winklhofer M, Hanzlik M (2010) Ant antennae: are they sites for magnetoreception? *J Roy Soc Interface* 7:143–152
- Riveros AJ, Srygley RB (2008) Do leaf-cutter ants *Atta colombica* orient their path-integrated, home vector with a magnetic compass? *Anim Behav* 75:1273–1281
- Rubens SM (1945) Cube-surface coil for producing a uniform magnetic field. *Rev Sci Instr* 16:243–246
- Seipke RF, Kaltenpoth M, Hutchings MI (2011) *Streptomyces* as symbionts: an emerging and widespread theme? *FEMS Microbiol Rev* 4: 862–876
- Vogt JT, Walleit B, Freeland TB Jr (2008) Imported fire ant (Hymenoptera:Formicidae) mound shape characteristics along a north–south gradient. *Environ Entomol* 37:198–205
- Vowles DM (1954) The orientation of ants. II. Orientation to light, gravity and polarized light. *J Exp Biol* 31:356–375
- Wajnberg E, Acosta-Avalos D, Alves OC, de Oliveira JF, Srygley RB, Esquivel DMS (2010) Magnetoreception in eusocial insects: an update. *J Roy Soc Interface* 7:S207–S225
- Walker MM, Bitterman ME (1985) Conditioned responding to magnetic fields by honeybees. *J Comp Physiol A* 157:67–71
- Wehner R, Michel B, Antonsen P (1996) Visual navigation in insects: coupling of egocentric and geocentric information. *J Exp Biol* 199: 129–140
- Wetterer J, Shafir S, Morrison L, Lips K, Gilbert G, Cipollini M, Blaney C (1992) On- and off-trail orientation in the leaf-cutting ant, *Atta cephalotes* L. (Hymenoptera Formicidae). *J Kansas Entomol Soc* 65:96–98
- Wiltschko R, Wiltschko W (1995) *Magnetic Orientation in Animals*. Springer, Berlin
- Winklhofer M (2010) Magnetoreception. *J Roy Soc Interface* 7:S131–S134
- Winklhofer M, Kirschvink JL (2010) A quantitative assessment of torque-transducer models for magnetoreception. *J Roy Soc Interface* 7:273–289
- Wirth R, Herz H, Ryel RJ, Beyschlag W, Hölldobler B (2003) Herbivory of leaf-cutting ants: a case study on *Atta colombica* in the tropical rainforest of Panama. Springer, Berlin
- Wittlinger M, Wehner R, Wolf H (2006) The ant odometer: stepping on stilts and stumps. *Science* 312:1965–1967
- Wohlgenuth S, Ronacher B, Wehner R (2001) Ant odometry in the third dimension. *Nature* 411:795–798
- Wolf H (2010) Odometry and insect navigation. *J Exp Biol* 214:1629–1641