



Do leafcutter ants, *Atta colombica*, orient their path-integrated home vector with a magnetic compass?

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Leafcutter ants, *Atta colombica*, forage over 250 m in structurally complex, Neotropical rainforests that occlude sun or polarized light cues. Night foraging makes the use of celestial cues and landmarks all the more difficult. We investigated the directional cues used by leafcutter ants to orient homeward by experimentally reversing the polarity of the local magnetic field and by experimentally subjecting the ants to a strong magnetic pulse to disrupt a magnetic compass. In both experiments, we transferred homeward-bound ants from a foraging trail to a table in a chamber that occluded landmark and celestial cues. In both experiments, control ants showed path integration and walked directly towards the nest. In the reversed field, one-half of the experimental ants oriented according to the reversed field (geographically 180° opposite to the nest's direction), indicating that they used a magnetic compass to update their positional reference derived from path integration. The other half walked towards the nest, suggesting that they may have used an egocentric reference to measure their rotation when displaced, although other explanations have not been entirely excluded. With application of a very brief, but strong, magnetic pulse, experimental ants oriented randomly. We conclude that the leafcutter ants use the earth's magnetic field as a reference by which to orient when path-integrating towards home.

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Central place foragers such as colonial social insects have multiple tools and strategies for orientation that can be divided into two basic categories. Egocentric cues include motion measured by proprioceptor sensory cells and visual input, which use the insect as a reference (Wehner et al. 1996; Collett & Collett 2000). Geocentric cues are landmarks, odours and other spatial features from which the insect is able to derive its current position relative to that of the nest and similar goals (Wehner et al. 1996).

Honeybees, *Apis mellifera*, and desert ants, *Cataglyphis* spp., integrate the distance and direction that they move through space to orient homeward (Wehner 1997).

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Celestial cues, including the sun's azimuth (Santschi 1911) and polarized skylight (von Frisch 1949), provide bees and ants with directional references (Labhart 1986; Rossel & Wehner 1986; Labhart & Meyer 2002). Without skylight, *Cataglyphis bicolor* workers orient randomly (Wehner 1982 cited in Wiltschko & Wiltschko 1995), and the availability of skylight might limit the activities of some ant species to daylight hours (e.g. *Cataglyphis cursor*). *Cataglyphis cursor* determines distance using proprioceptive information as evident from experiments in darkness (Thiélin-Bescond & Beugnon 2005). Horizon and canopy landmarks can also provide geocentric cues for ants (Hölldobler 1980; Fukushi 2001; Fukushi & Wehner 2004; Andel & Wehner 2004).

The ability to return home under overcast skies and under nonpolarized light indicates that at least some ants do not require celestial or landmark cues to path-integrate (e.g. the grass ant, *Tetramorium caespitum*; Ronacher et al. 2001). Other arthropods, like the bumblebee, *Bombus*

impatiens (Chittka et al. 1999), and the wandering spider, *Leucorchestris arenicola* (Norgaard 2005; Norgaard et al. 2006), are capable of homing in complete darkness even in the absence of odour cues, suggesting a role for a combination of proprioceptive and magnetic cues. A magnetic compass has been shown for wood ants, *Formica rufa* (Camlitepe & Stradling 1995), and weaver ants, *Oecophylla smaragdina* (Jander & Jander 1998). Banks & Srygley (2003) found that leafcutter ants, *Atta colombica*, responded to polar-reversal of the local magnetic field when the sky was overcast, but instead of the predicted shift in orientation of 180°, the experimental ants shifted their mean orientation only 74° relative to that of the control ants, which oriented homeward in a natural magnetic field. Additional evidence for a magnetic sense as a compass comes from the study of magnetic particles in the migratory ant *Pachycondyla marginata* (Wajnberg et al. 2004; Acosta-Avalos et al. 1999), which orients north or south along the geomagnetic field (Acosta-Avalos et al. 2001). However, the role of the magnetic compass in path integration has, to our knowledge, never been shown in any arthropod.

Neotropical leafcutter ants, *A. colombica* (Formicidae: Atini), forage over great expanses by following pheromonally marked and architecturally modified foraging trails. They climb on trees and smaller plants to cut leaves and carry the leaf tissue to their subterranean fungal gardens (Wirth et al. 2003). Thus they are not like desert ants, which forage solitarily by circuitous routes over vast distances in habitats with little vegetative structure and then return directly homeward (Andel & Wehner 2004; Narendra 2007).

Leafcutter ants are active when and where skylight and the sun are not visible. They forage in the deep shade of the forest understory, in overcast conditions or during night. For this reason, we investigated whether leafcutter ants use a magnetic compass to orient and path-integrate. We experimentally reversed the polarity of the local magnetic field while the ants were foraging nocturnally to elicit a reversal in their path-integrated orientation without the use of landmarks or celestial cues. In a second experiment, we experimentally disrupted the ants' magnetic compass with a strong magnetic pulse to elicit a change in their ability to path-integrate towards the nest. In both experiments, we found that when displaced from the trail, leafcutter ants are similar to desert ants in that they orient directly towards the nest. However, unlike desert ants, in the absence of other cues, leafcutter ants *A. colombica* rely on a magnetic compass to determine a vector home during path integration.

METHODS

Experimental Animals and Study Sites

We conducted the experiments using mature colonies of *Atta colombica* Guérin-Ménéville, which had nests with at least five entrance holes and all castes, including large soldiers. For both experiments, we used bait to divert foraging trails and we selected ants of similar size carrying the

bait back to the nest, excluding larger soldier ants and smaller ants, which do not carry leaf cuttings.

The experiments were conducted in two rainforest areas in Panama. During November 2002, we studied a colony on Barro Colorado Island (BCI) (elevation 40 m; 9°09'N, 79°1'W). This colony foraged both diurnally (starting around 0900 hours) and nocturnally (sometimes until 0300 or 0400 hours) and was used in the experiments of reversal of the ambient magnetic polarity (see below). A second colony, studied during July 2006 was located in a forest understory in Gamboa (elevation 30 m; 9°07'N, 79°42'W). This colony was used in the experiments of disruption of the magnetic compass (see below).

Experimental Manipulations and Analysis

Experimental reversal of the ambient magnetic polarity

We focused on evening and nocturnally foraging ants collecting food after 1730 hours when the sun was not visible from the experimental site. First, we diverted a foraging trail of an ant colony (Fig. 1a, b) by scattering oat flakes in a line to a large Merritt four-coil electromagnet (Merritt et al. 1983) where the horizontal component of the magnetic field could be experimentally reversed. Details of the magnetic conditions inside the electromagnet and at the study site are described by Srygley et al. (2006).

The magnet was enshrouded with a black nylon tarp covered with black vinyl, and the construction was under a translucent white nylon-reinforced plastic tent (6 m long, 3.4 m wide, 2–4 m high, peaking at the central north–south line, closed to all sides and above; Larin Corp., Ontario, Canada). Inside the coil, we levelled a wooden table (70 × 70 cm) on a reinforced cardboard box and a 19-litre plastic tub so that it was at the vertical centre of the coil (1.2 m above the ground). The table was covered with sand and the centre of the table marked with a fingertip depressed into the sand. The sand was replaced between trials to discard any pheromonal cues.

We collected an individual by gripping the oat flake that the ant was carrying after it began to walk north along the foraging trail back to the nest (Fig. 1b, c). Thus the ant was not seized or wounded in any way. The ant was carried 1.25 m due south into the completely darkened electromagnet (0 lx; Extech digital light meter, Model 401025; measured every new experimental day) and oriented in no particular direction when set at the centre of the wooden table as judged from the camera image. The table centre corresponded with the centre of the electromagnet (i.e. 1.2 m from each side; Fig. 1c). The ant was tested in one of two treatments randomly selected by coin toss. The control group was tested in the natural, geomagnetic field (which in Panama is oriented towards geographical north), and the experimental group was tested in a magnetic field of reversed polarity (i.e. horizontal component of the magnetic field locally shifted 180°; all directions throughout the paper are given relative to geographical north). We tracked the movement of the ant by using an infrared camcorder (Sony TRW-700; taping at 30 frames/s and left on until all ants were treated for the

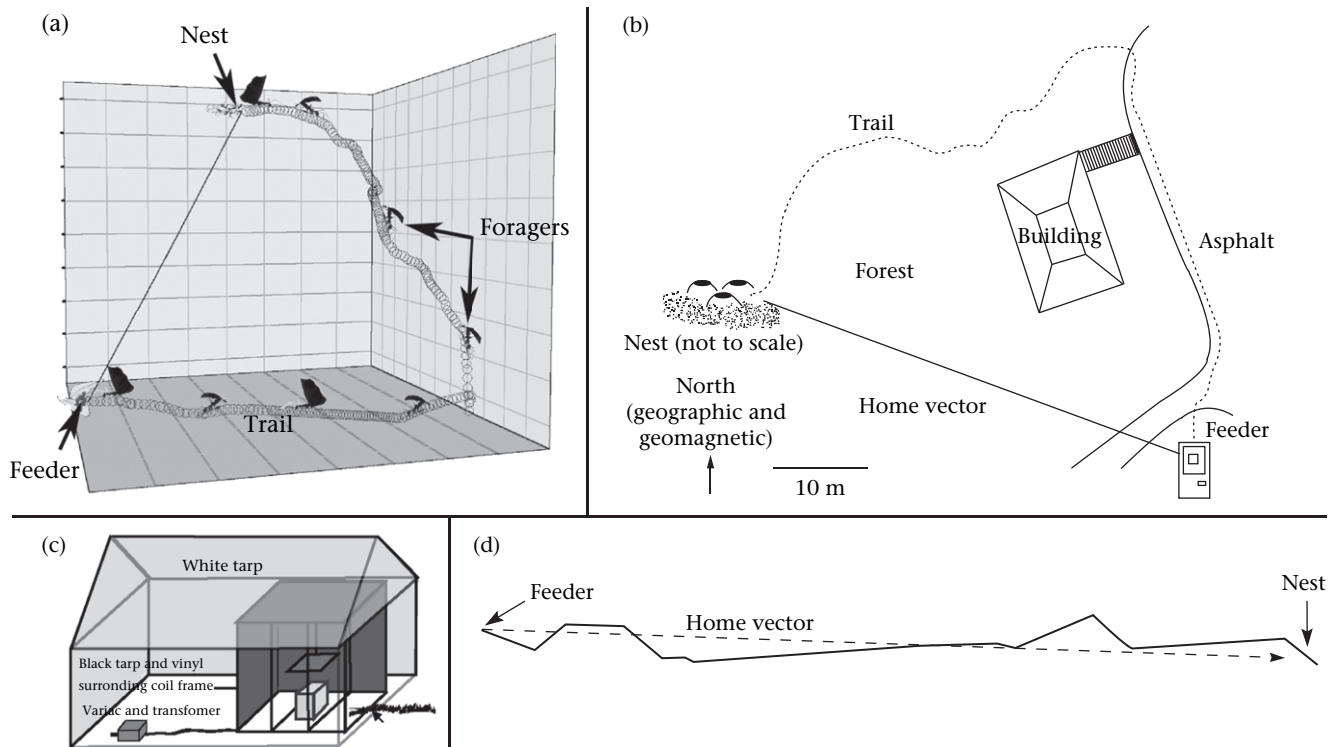


Figure 1. (a) Three-dimensional reconstruction of the foraging trail between the feeder and the nest during the reversal experiment. Each circle represents a measure of inclination every 50 cm. (b) Foraging trail and ‘bee-line’ home direction of *Atta colombica* between the electromagnet and the nest during the reversal experiment. The dotted line is the foraging trail. The home direction (296°) points from the orientation chamber to the nest. The compass points to north (0°), which is the direction that the foraging column departed with oat flakes to return to the nest. (c) Perspective drawing of the orientation table within the Merritt four-coil magnet and the sheltering tarps. The location where the ant was removed from the foraging trail is indicated by the arrow. (d) Foraging trail and bee-line home direction of *A. colombica* between the circular arena and the nest during the experiment to disrupt the magnetic compass.

night) hand-held from the northeast corner of the electromagnet. The ant’s coordinates on the table were recorded when it approached the edge of the table (i.e. ~ 33 cm from the centre) and later transformed to compass angle by an observer unaware of the treatment.

We tested 124 ants, excluding seven that dropped the oat flake and walked in circles. To test whether light conditions outside the arena affected the ants’ orientations, we categorized skylight into one of four conditions (for definitions, see Table 1 footnotes): daylight prior to civil twilight, between civil and nautical twilight, between nautical and astronomical twilight, and starlight (there was no moonlight during testing). To meet the assumption that expected frequencies are at least five for chi-square tests of circular data (Batschelet 1981, page 110), we combined ant orientations into one of two hemispheres (north and south) and compared orientations before astronomical twilight to those under starlight. These were not significantly different (chi-square test: $\chi_1^2 = 3.08$, $P = 0.079$). For subsequent tests, we used all of the data collected for each treatment.

The significance of the circular distributions in each treatment was tested with the Rayleigh test (software Oriana ver 2.02; Batschelet 1981). We compared the mean directions of our distributions with the predicted orientation towards home (path-integrated direction) and another departing the electromagnet northward

along the foraging trail with V tests. The distributions of orientations in each treatment were compared with chi-square (Batschelet 1981) and Watson’s U^2 tests (Zar 1999).

Experimental disruption of the magnetic compass

At approximately 40 m from the nest, we diverted a foraging trail by scattering barley flakes in a line for 2.8 m to approximately 1 m from the centre of an orientation arena (Fig. 1d). Once trained to a pile of barley flakes each day, the ants continued to collect and carry the flakes to the nest throughout the experiment. The orientation arena was a round plastic aquarium lid, 60 cm inside and 61.5 cm outside diameter, which was levelled on the ground and filled with sand. A round opaque plastic table was placed above the arena to block the view of the forest canopy and sky. A white translucent cotton sheet surrounded the arena to block the horizon and potential landmarks.

An ant that had picked up a barley flake and returned ca. 5 cm down the trail to the nest was collected with forceps by handling the flake so that the ant was not handled directly. It was carried 2.0 m to an electric coil through which a weak current passed to produce a weak magnetic field (the biasing field: 0.38 mT) oriented on a north–south axis. Each ant was treated one of three ways. Controls were placed in the weak magnetic field oriented

Table 1. Counts of ants orienting towards four compass quadrants with the light conditions outside the arena and the experimental magnetic condition inside

	0–89°	90–179°	180–269°	270–359°
Natural (coil off)				
Daylight (solar disc obstructed)	2	1	3	4
Civil–nautical twilight*	0	0	0	4
Nautical–astronomical twilight†	0	0	1	0
Starlight	14	7	14	17
Total	16	8	18	25
Polarity reversed (coil on)				
Daylight (solar disc obstructed)	0	1	0	6
Civil–nautical twilight	0	0	0	0
Nautical–astronomical twilight	0	0	0	3
Starlight	6	17	6	11
Total	6	18	6	20

*Civil twilight, when sunlight was barely perceptible and details of objects on the ground were no longer visible (1818 hours); nautical twilight, when outlines of objects on the ground could no longer be distinguished by humans (1843 hours) data from U.S. Naval Observatory Astronomical Applications Department <http://aa.usno.navy.mil/>.

†Astronomical twilight, when the sun no longer contributed to illumination of the atmosphere over BCI (1908 hours).

with their heads towards geomagnetic north and towards the northern end of the bias field for 7 s. North-biased ants were placed in the weak magnetic field as were the controls and a magnetic pulser (Sota model MPG5; modified by Kirschvink (1983), California Institute of Technology, to have a weak biasing field for which the orientation was reversible with a switch) was charged over 7 s with a 12 V battery and then an electric current was released into a coil such that the ants were subjected to a strong magnetic field of brief duration (55 mT for 2 ms) oriented in a direction opposite to the weak biasing field (hereafter 'antiparallel treatment'). South-biased ants were held in a weak magnetic field of identical strength but oriented 180° opposite to the fields for the control and north-biased ants. The ants were subjected to the same strong brief magnetic pulse but they were pulsed parallel to the biasing field (hereafter 'parallel treatment'). Following treatment, the ant was placed in the centre of the arena and filmed from above with a Sony digital video camera mounted on the table so that the lens filled the centre hole, 80 cm above the arena. The coordinate where the ant crossed a circle 2 cm within the edge of the arena was converted into an angular orientation relative to the centre of the arena. The time that the ant required to reach this circle was also measured. The sand was replaced between trials to avoid the use of pheromonal cues.

The predicted results of pulse magnetization depend on the materials and construction of the compass (Wiltschko & Wiltschko 2005). The strength of the magnetic pulser (55 mT) should be sufficient to overcome the coercivity of single-domain biological magnetite crystals. The coercivity of single-domain magnetite in rainbow trout is between 20 and 40 mT (Diebel et al. 2000). Magnetotactic bacteria have single-domain magnetite particles with coercivities ranging from 30 to 45 mT (Dunin-Borkowski et al. 1998). The biasing field serves to align the magnetite particles in the same direction prior to applying the pulse. For single-domain magnetite (ranging in size from 30 to 200 nm, depending on the shape of the particles: Butler

& Banerjee 1975; Kirschvink & Hagadorn 2000), a magnetic pulse to the magnetite in a parallel biasing field would not result in a change in the magnetic spin within the crystals and the animals' orientations would not be different from that of the controls, whereas a magnetic pulse in an antiparallel biasing field would result in an orientation opposite to that of the controls (Kirschvink & Kobayashi 1991; Wiltschko et al. 2002). If the magnetite crystals are larger than the critical size for single-domain magnetite, then they organize into domains with a magnetic moment that differs in direction depending on the domain. Remanence and coercivity are affected by the motion of the domain wall, whereas remagnetization of single-domain particles results from rotation of the magnetic moment (Banerjee & Moskowitz 1985). With application of a magnetic pulse, multidomain particles would change their magnetization. However, the predicted change in orientation would differ from one subject to the next pulse such that the experimental group could be randomly oriented. If the magnetite crystals are small enough, thermal fluctuations can be sufficient to cause an unstable magnetization. However, they can align to an external magnetic field and thus are called superparamagnetic. A noninteracting system of superparamagnetic particles would rapidly relax to its equilibrium condition following a pulse, but a cluster of superparamagnetic particles might be disrupted by a magnetic pulse when applied at an angle greater than 45° relative to the chain-like organization of the cluster (Davila et al. 2005). In this latter case, the animals would orient randomly in the absence of other cues. Although the cluster might reorganize, it would require a few hours to several days (Davila et al. 2005).

The significance of the circular distributions of the ants in each treatment was tested with the Rayleigh test. We compared the mean directions of our distributions with the predicted path-integrated orientation towards home with *V* tests. The distributions of orientations in each treatment were compared with chi-square tests (Batschelet 1981).

RESULTS

Experimental Reversal of the Ambient Magnetic Polarity

The control group was significantly oriented towards the predicted path-integrated home direction (Rayleigh test: $\mu = 294^\circ$, $r = 0.26$, $N = 67$, $P = 0.01$), although we were unable to distinguish whether *A. colombica* adjusted for the 5° difference resulting from vertical displacement when path-integrating (*V* tests: $P < 0.005$ relative to the nest direction with or without adjustment for elevational changes; Fig. 2a). The ants did not orient towards the foraging trail departing the electromagnet northward (0° ; *V* test: $\mu = 1.239$, $P = 0.108$).

The experimental group was oriented axially (i.e. in two directions: 127° and 307° , on average; Fig. 2b; Rayleigh

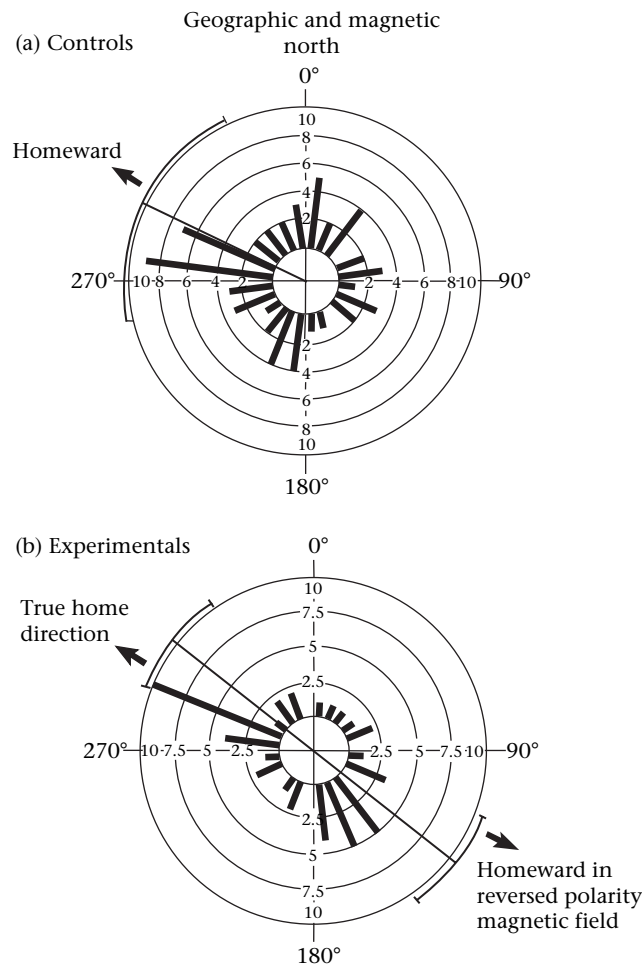


Figure 2. Circular histograms of ant orientations (a) under natural magnetic polarity (coil off, $N = 67$) and (b) when magnetic north was reversed to geographical south (coil on, $N = 50$). The foraging trail back to the nest is towards geographical north (0°), and the bee-line vector to the nest is towards 296° . The radius represents the direction of the mean vector in (a) 294° , and the equatorial line is the mean orientation axis in (b) 127° and 307° . The arc outside the circle is the 95% confidence interval for the mean vector or axis. Values inside the circles are the number of individuals.

test: $r = 0.33$, $N = 50$, $P < 0.01$). One direction did not differ significantly from that towards the nest (i.e. refer to confidence intervals in Fig. 2b), whereas the other was in the predicted direction if ants used a magnetic compass to orient.

Ant orientations differed significantly between treatments when classified into four compass quadrants (chi-square test: $\chi^2_3 = 18.5$, $P < 0.001$; Table 1). The difference between treatments was also evident in the qualitative inequalities found between the distributions (i.e. vectorial versus axial, respectively). In addition to this fundamental discrepancy, which supports the effect of the treatment but implicitly limits the statistical comparison, the distributions of orientations in the treatments were statistically different by Watson's test ($U_{50,67}^2 = 1.339$, $P < 0.001$).

The mean \pm SD times for the ants to walk to the edge of the table were 75.4 ± 41.3 s for the control ants and 75.2 ± 49.0 s for the experimental ants. Those experimental ants that walked towards the predicted nest direction under the reversed magnetic field (i.e. directions between 27° and 206°) reached the edge of the table in 70.5 ± 42.4 s, whereas those that walked towards the true nest direction reached the edge in 79.2 ± 54.4 s. Neither of these temporal comparisons differed significantly (Student's *t* tests: time was log-transformed to meet the assumptions of normality: control ants versus experimental ants: $t_{115} = 0.51$, $P = 0.61$; experimental ants towards predicted versus towards true nest direction: $t_{48} = 0.72$, $P = 0.47$).

Experimental Disruption of the Magnetic Compass

The control group oriented significantly (Rayleigh test: $\mu = 136^\circ$, $r = 0.31$, $N = 49$, $P = 0.01$; Fig. 3a) towards the predicted path-integrated home direction, 92° (*V* test: $\mu = 0.214$, $P = 0.017$). The Antiparallel group and the Parallel group were each dispersed randomly (Rayleigh test: $r = 0.13$ for each treatment, $N = 47$ in each treatment, $P = 0.47$ and $P = 0.45$, respectively; Fig. 3b, c). Their distributions did not differ significantly (chi-square test: $\chi^2_5 = 0.96$, $P > 0.50$; Table 2), and so we pooled the two groups. The combined pulse magnet treatment group was dispersed randomly (Rayleigh test: $\mu = 158^\circ$, $r = 0.13$, $N = 94$, $P = 0.21$; Fig. 3d), and it was not oriented homeward (*V* test: $\mu = 0.05$, $P = 0.26$).

The time for each ant to walk to the edge of the arena was cube-root transformed to normalize the distributions of the data within treatments. Times to the edge were 58 ± 44 s for the control ants and 45 ± 29 s for the experimental ants (means \pm SD), but the means did not differ significantly (Student's *t* test: $t_{141} = 1.73$, $P = 0.085$).

DISCUSSION

Here we have shown that foragers of the leafcutter ant *A. colombica* can rely on magnetic information to orient their path-integrated home vector. In our first experiment, night-foraging workers responded to a local reversal in the polarity of the geomagnetic field. The response of

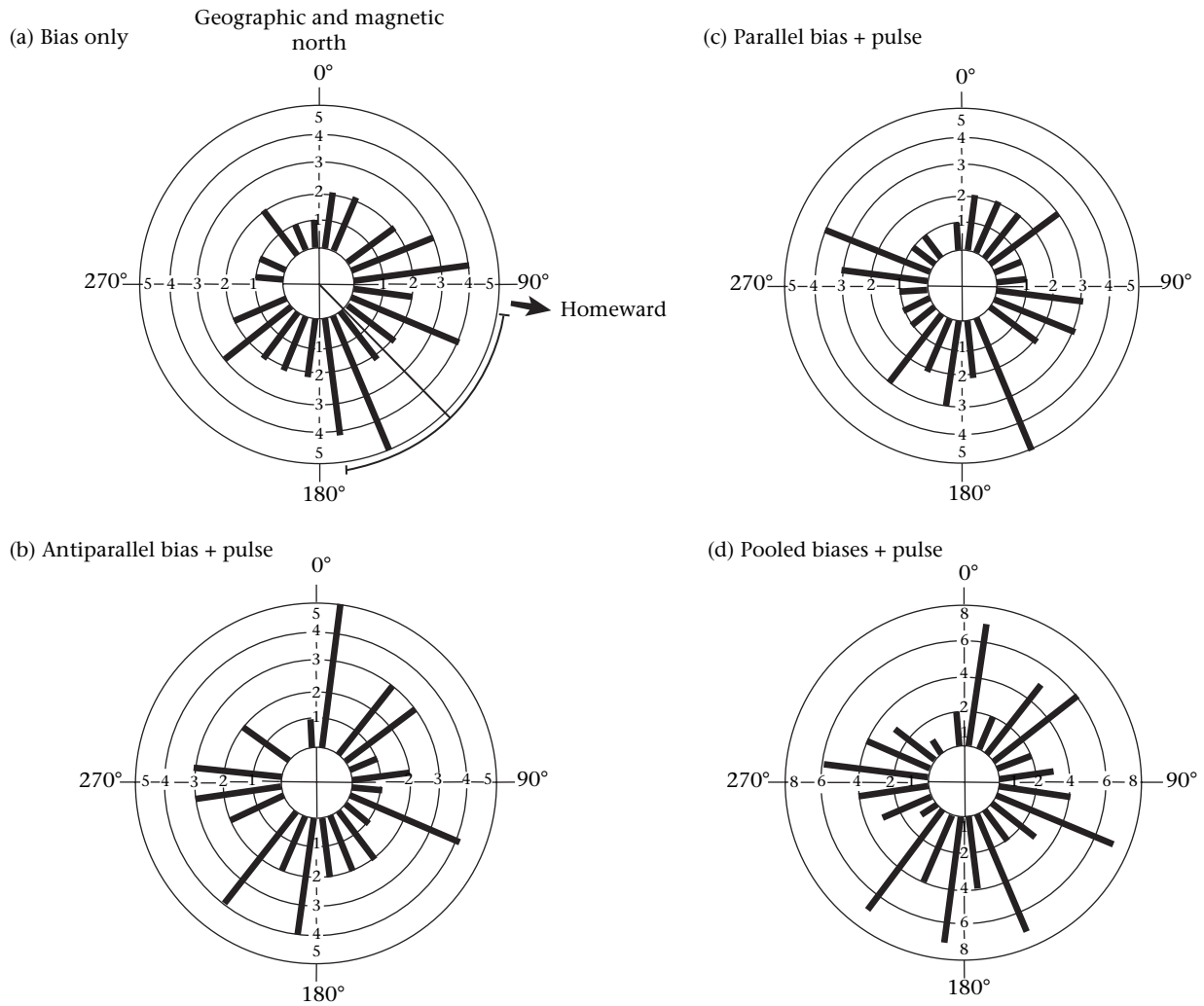


Figure 3. Circular histograms of ant orientations after exposure to (a) a biasing field (control ants), (b) an antiparallel bias plus a magnetic pulse and (c) a parallel bias plus a magnetic pulse. (d) Orientation of the ants including data pooled from antiparallel and parallel biases. The radius and arc represent the mean vector and its 95% confidence interval. Values inside the circles are the number of individuals.

approximately one-half of the experimental ants was in the predicted direction towards the nest in a reversed magnetic field. Furthermore, workers foraging during overcast days oriented randomly after exposure to a strong magnetic pulse, demonstrating a disturbance of the path-integrated vector calculation. From this evidence, we conclude that the ants used a magnetic compass to orient their path-integrated home vector.

Table 2. Counts of experimentally treated ants orienting towards six compass segments

	1– 60°	61– 120°	121– 180°	181– 240°	241– 300°	301– 360°
North-bias pulse group	10	7	8	9	8	5
South-bias pulse group	9	8	10	8	9	3
Combined pulse groups	19	15	18	27	17	8
Controls	5	13	13	9	4	5

Nevertheless, during the reversal experiment, the control ants and approximately one-half of the experimental ants oriented directly towards the true nest position. In a second colony of day-foraging *A. colombica*, ants also path-integrated towards the home direction (Banks & Srygley 2003). However, in these daytime-foraging ants, a hierarchy of cues was evident with the sun compass given greater weight than the magnetic compass. In our study, the ants were prevented from orienting with celestial cues and other visual cues either by being tested in a completely darkened environment (electromagnet during reversal treatment), during hours of dim light (night and overcast days) or by directly occluding landmarks (pulse experiment). When local path-integrated vectors and landmarks (Schatz et al. 1999) are prevalent orientation cues, desert ants *Cataglyphis fortis* inhibit the global path-integrated vector home (Collett et al. 1998). In contrast, when landmarks are eliminated, desert ants rely on the global vector (Andel & Wehner 2004). For *A. colombica*, the apparent use of egocentric cues to path-integrate homeward by the control ants and half of the

experimental ants in the reversed magnetic field probably resulted from our experimental design, which obstructed landmarks and discarded pheromonal cues. In nature, ants might use path integration to orient homeward when exploring new foraging sites, when straying from trails or when trails are washed away by sudden downpours. Although not demonstrated here, compensating the path-integrated vector for elevational changes would allow the ants to orient towards the nest when they accidentally fall from canopy vegetation.

Some experimental ants oriented towards the true home direction during the reversal of the magnetic field despite the experimental manipulation and the lack of landmarks and celestial cues with which to orient. They oriented towards home even though each ant was lifted from the foraging trail and placed on the table such that its starting orientation was random. Three cues may have oriented them towards home: odours, subterranean-borne sound and proprioceptors. First, odour would not indicate home to within 15° at a straight-line distance of 50 m to the nest. An odour gradient may have provided sufficient information to orient the ants towards the home semicircle (Jaffe et al. 1990) and the magnetic axis given the precise direction within the semicircle. Second, leaf-cutting ants are extremely sensitive to vibration (Roces & Tautz 2001), but seismic sounds conveyed to the table support would have been dampened by the cardboard box, plastic tub, wooden table and sand layer on the table top. Although we do not believe that vibration would indicate home to within 15° in our experimental design, the role of seismic sound as an orientation cue warrant further investigation. Third, the ants may have used egocentric cues to determine the home vector. Because the ants lost contact with the surface when transferred to the table, the direction cue cannot be based on walking or other idiothetic cues. Rather, direction may have been indicated by proprioceptors that measure movement through space. However, a statocyst-like sensory mechanism is known only in aquatic insect larvae and adult Ephemeroptera (Horn 1985). The body may act like a statolith whose relative inertia stimulates proprioceptors in the extremities (Lindauer & Nedel 1959).

Also, proprioception was probably involved in the calculation of the path-integrated vector, because the ants had to compensate for elevation distances, accounting solely for horizontal displacements. The desert ants (*Cataglyphis* spp.) use idiothetic cues derived from their own movements and from gravity to adjust the path-integrated direction for vertical displacement (Wohlgemuth et al. 2001). If the leafcutter ants possess such a system, then they would discount 20 m of the total 108 m distance along the trail between the nest and the electromagnet as a result of elevational changes in microhabitat (measured every 0.5 m; Fig. 1a). The difference between the adjusted value (296°) and that without correcting for vertical displacement (291°) was only 5° and could not be detected from our statistical analysis. We think that the proprioceptive abilities of leafcutter ants to measure rotation in the absence of visual cues and compensate for elevation displacements warrants further investigation.

For the experimental group, in the reversal experiment, one-half of the ants reoriented their home vector in line with the change in magnetic polarity. In all cases, the reversed magnetic polarity caused the ants' compass to indicate a direction 180° opposite to the path-integrated direction homeward. For the subset that reoriented away from the nest, this realignment serves as the first evidence that ants use their magnetic compass to reorient a path-integrated home vector. Because proprioceptors serve as independent measures of angular change by which to path-integrate the home vector, the ants used their magnetic compass to adjust directional information gained from proprioceptors. This is further supported by our results of the pulse experiment; workers exposed to a strong magnetic pulse oriented randomly in contrast to the path-integrating workers of the control group. Therefore, we conclude that leafcutter ant workers also used a magnetic compass to orient their path-integrated home vector.

Interestingly, the results from our pulse experiment also provide insight on the composition of the magnetic compass. As described above (see *Methods*), a compass based on single-domain magnetite should not be affected by the magnetic pulse following the ant's exposure to the parallel-biased field and the orientations would not be different from those in the control group. Also, a reversal in orientation would be expected from the magnetic pulse after exposure to the antiparallel field. In contrast, if the compass were composed of multidomain or superparamagnetic particles, the ants might be oriented randomly as a result of exposure to the magnetic pulse independent of the biasing field. The complete disruption of the ants' orientation after exposure to any of the treatments (Fig. 3b, c, d) supports the presence of a compass based on multidomain and/or superparamagnetic particles rather than on single-domain particles.

Together, our results show the use of a magnetic compass during path-integrated navigation in leafcutter ants and provide insights on the nature of the particles forming the compass. This is the first evidence of the magnetic compass being involved in path integration in an invertebrate.

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References

- Acosta-Avalos, D., Wajnberg, E., Oliveira, P. S., Leal, I. I., Farina, M. & Esquivel, D. M. 1999. Isolation of magnetic nanoparticles from *Pachycondyla marginata* ants. *Journal of Experimental Biology*, **202**, 2687–2692.
- Acosta-Avalos, D., Esquivel, D. M., Wajnberg, E., de Barros, H. G., Oliveira, P. S. & Leal, I. 2001. Seasonal patterns in the orientation system of the migratory ant *Pachycondyla marginata*. *Naturwissenschaften*, **88**, 343–346.
- Andel, D. & Wehner, R. 2004. Path integration in desert ants, *Cataglyphis*: how to make a homing ant run away from home. *Proceedings of the Royal Society of London Series B*, **271**, 1485–1489.
- Banerjee, S. K. & Moskowitz, B. M. 1985. Ferrimagnetic properties of magnetite. In: *Magnetite Biomineralization and Magnetoreception in Organisms: a New Biomagnetism (Topics in Geobiology)* (Ed. by J. L. Kirschvink, D. S. Jones & B. J. MacFadden), pp. 17–41. New York: Plenum.
- Banks, A. N. & Srygley, R. B. 2003. Orientation by magnetic field in leaf-cutter ants, *Atta colombica* (Hymenoptera: Formicidae). *Ethology*, **109**, 835–846.
- Batschelet, E. 1981. *Circular Statistics in Biology*. London: Academic Press.
- Butler, R. F. & Banerjee, S. K. 1975. Theoretical single-domain grain size range in magnetite and titanomagnetite. *Journal of Geophysical Research*, **80**, 4049–4058.
- Camlitepe, Y. & Stradling, D. 1995. Wood ants orient to magnetic fields. *Proceedings of the Royal Society of London, Series B*, **261**, 37–41.
- Chittka, L., Williams, N. M., Rasmussen, H. & Thomson, J. D. 1999. Navigation without vision: bumblebee orientation in complete darkness. *Proceedings of the Royal Society of London, Series B*, **266**, 45–50.
- Collett, M. & Collett, T. S. 2000. How do insects use path integration for their navigation? *Biological Cybernetics*, **83**, 245–259.
- Collett, M., Collett, T. S., Bisch, S. & Wehner, R. 1998. Local and global vectors in desert ant navigation. *Nature*, **394**, 269–272.
- Davila, A. F., Winklhofer, M., Shcherbakov, V. P. & Petersen, N. 2005. Magnetic pulse affects a putative magnetoreceptor mechanism. *Biophysical Journal*, **89**, 56–63.
- Diebel, C. E., Proksch, R., Green, C. R., Neilson, P. & Walker, M. M. 2000. Magnetite defines a vertebrate magnetoreceptor. *Nature*, **406**, 300–302.
- Dunin-Borkowski, R. E., McCartney, M. R., Frankel, R. B., Bazylinski, D. A., Pósfai, M. & Busek, P. R. 1998. Magnetic microstructure of magnetotactic bacteria by electron holography. *Science*, **282**, 1868–1870.
- von Frisch, K. 1949. Die Polarisation des Himmelslichtes als orientierender Faktor bei den Tänzern der Bienen. *Experientia*, **5**, 142–148.
- Fukushi, T. 2001. Homing in wood ants, *Formica japonica*. *Journal of Experimental Biology*, **204**, 2063–2072.
- Fukushi, T. & Wehner, R. 2004. Navigation in wood ants, *Formica japonica*: context dependent use of landmarks. *Journal of Experimental Biology*, **207**, 3431–3439.
- Hölldobler, B. 1980. Canopy orientation: a new kind of orientation in ants. *Science*, **210**, 86–88.
- Horn, E. 1985. Gravity. In: *Nervous System: Sensory. Vol. 6, Comprehensive Insect Physiology: Biochemistry and Pharmacology* (Ed. by G. A. Kerkut & L. I. Gilbert), pp. 557–576. Exeter: Pergamon.
- Jaffe, K., Ramos, C. & Lagalla, C. 1990. Orientation cues used by insects. *Insectes Sociaux*, **37**, 101–115.
- Jander, R. & Jander, U. 1998. The light and the magnetic compass of the weaver ant, *Oecophylla smaragdina* (Hymenoptera: Formicidae). *Ethology*, **104**, 743–758.
- Kirschvink, J. L. 1983. Biogenic ferrimagnetism: a new biomagnetism. In: *Biomagnetism: an Interdisciplinary Approach* (Ed. by S. J. Williamson, R. Gian-Luca, K. Lloyd & I. Modena), pp. 501–532. New York: Plenum.
- Kirschvink, J. L. & Hagadorn, J. W. 2000. A grand unified theory of biomineralization. In: *The Biomineralisation of Nano- and Micro-structures* (Ed. by E. Bäuerlein), pp. 139–150. Weinheim, Germany: Wiley-VCH Verlag GmbH.
- Kirschvink, J. L. & Kobayashi, A. 1991. Is geomagnetic sensitivity real? Replication of the Walker–Bitterman magnetic conditioning experiments in honey bees. *American Zoologist*, **31**, 169–185.
- Labhart, T. 1986. The electrophysiology of photoreceptors in different eye regions of the desert ant, *Cataglyphis bicolor*. *Journal of Comparative Physiology A*, **158**, 1–7.
- Labhart, T. & Meyer, E. P. 2002. Neural mechanisms in insect navigation: polarization compass and odometer. *Current Opinion in Neurobiology*, **12**, 707–714.
- Lindauer, M. & Nedel, J. O. 1959. Ein Schweresinnesorgan der Honigbiene. *Zeitschrift für Vergleichende Physiologie*, **42**, 334–364.
- Merritt, R., Purcell, C. & Stroink, G. 1983. Uniform magnetic field produced by three, four, and five square coils. *Review of Scientific Instruments*, **54**, 879–882.
- Narendra, A. 2007. Homing strategies of the Australian desert ant *Melophorus bagoti* II. Interactions of the path integrator with visual cue information. *Journal of Experimental Biology*, **210**, 1804–1812.
- Norgaard, T. 2005. Nocturnal navigation in *Leucorchestris arenicola* (Araneae, Sparassidae). *Journal of Arachnology*, **33**, 533–540.
- Norgaard, T., Henschel, J. R. & Wehner, R. 2006. The night-time temporal window of locomotor activity in the Namib Desert long-distance wandering spider, *Leucorchestris arenicola*. *Journal of Comparative Physiology A*, **192**, 365–372.
- Roces, F. & Tautz, J. 2001. Ants are deaf. *Journal of the Acoustical Society of America*, **109**, 3080–3082.
- Ronacher, B., Gallizzi, K., Wohlgenuth, S. & Wehner, R. 2001. Lateral optic flow does not influence distance estimation in the desert ant *Cataglyphis fortis*. *Journal of Experimental Biology*, **203**, 1113–1121.
- Rossel, S. & Wehner, R. 1986. Polarization vision in bees. *Nature*, **323**, 128–131.
- Santschi, F. 1911. Observations et remarques critiques sur le mécanisme de l'orientation chez les fourmis. *Revue Suisse de Zoologie*, **19**, 305–338.
- Schatz, B., Chameron, S., Beugnon, G. & Collett, T. S. 1999. The use of path integration to guide route learning in ants. *Nature*, **399**, 769–772.
- Srygley, R. B., Dudley, R., Oliveira, E. G. & Riveros, A. J. 2006. Experimental evidence for a magnetic sense in Neotropical migrating butterflies (Lepidoptera: Pieridae). *Animal Behaviour*, **71**, 183–191.
- Thiélin-Bescond, M. & Beugnon, G. 2005. Vision-independent odometry in the ant *Cataglyphis cursor*. *Naturwissenschaften*, **92**, 193–197.
- Wajnberg, E., Cernicchiaro, G. & Esquivel, D. M. 2004. Antennae: the strongest magnetic part of the migratory ant. *Biomaterials*, **17**, 467–470.
- Wehner, R. 1997. The ant's celestial compass system: spectral and polarization channels. In: *Orientation and Communication in Arthropods* (Ed. by M. Lehrer), pp. 145–185. Basel: Birkhäuser-Verlag.
- Wehner, R., Michel, B. & Antonsen, P. 1996. Visual navigation in insects: coupling of egocentric and geocentric information. *Journal of Experimental Biology*, **199**, 129–140.
- Wiltschko, R. & Wiltschko, W. 1995. *Magnetic Orientation in Animals*. Berlin: Springer-Verlag.

- Wiltschko, W. & Wiltschko, R. 2005. Magnetic orientation and magnetoreception in birds and other animals. *Journal of Comparative Physiology A*, **191**, 675–693.
- Wiltschko, R., Munro, U., Wiltschko, R. & Kirschvink, J. L. 2002. Magnetite-based magnetoreception in birds: the effect of a biasing field and a pulse on migratory behavior. *Journal of Experimental Biology*, **205**, 3031–3037.
- Wirth, R., Herz, H., Ryel, R. J., Beyschlag, W. & Hölldobler, B. 2003. *Herbivory of leaf-cutting ants: a case study on Atta colombica in the tropical rainforest of panama*. *Ecological Studies*. Vol. 164. Berlin: Springer.
- Wohlgemuth, S., Ronacher, B. & Wehner, R. 2001. Ant odometry in the third dimension. *Nature*, **411**, 795–798.
- Zar, J. H. 1999. *Biostatistical Analysis*. Upper Saddle River, New Jersey: Prentice Hall.