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ISOLATION OF MAGNETIC NANOPARTICLES FROM *PACHYCONDYLA MARGINATA* ANTS

DANIEL ACOSTA-AVALOS[1], ELIANE WAJNBERG[1], PAULO S. OLIVEIRA[2], INARA LEAL[2], MARCOS FARINA[3] AND DARCI M. S. ESQUIVEL[1,*]

[1]*Centro Brasileiro de Pesquisas Físicas, Rua Xavier Sigaud 150, 22290–180, Rio de Janeiro, Brazil,* [2]*Departamento de Zoologia, Universidade Estadual de Campinas, Campinas, CP 6109, 13083–970 Campinas, São Paulo, Brazil* and [3]*Departamento de Anatomia, Centro de Ciencias da Saude, Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941–590, Brazil*

*Author for correspondence at present address: Departamento de Materia Condensada e Espectroscopia, Centro Brasileiro de Pesquisas Físicas, Rua Xavier Sigaud 150, 22290–180, Rio de Janeiro, Brazil (e-mail: darci@cbpf.br)

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Summary

We report on the presence of magnetic iron oxides in the migratory ant *Pachycondyla marginata*. Magnetic particles were extracted from different parts of the ant (head, thorax and abdomen) using magnetic precipitation methods. Electron spectroscopic images for iron and oxygen were obtained from the extracted particles, and, by using the corresponding electron micrographs, histograms of size distribution were constructed. Selected area diffraction patterns were also obtained from the particles, and analysis of these showed the presence of a mixture of different iron oxides, including the magnetic oxides, magnetite and maghemite. The size distribution of the particles in the abdomen is different from that in the thorax and the head. In accordance with the hypothesis of magnetic orientation based on the presence of magnetic material within the body, two regions of the ant, the head and the abdomen, could be implicated in the detection of the geomagnetic field.

Key words: migratory ant, *Pachycondyla marginata*, magnetoreception, iron oxide, electron microscopy, electron diffraction, biomineralization.

Introduction

It is known that the behaviour of a great variety of higher animals is influenced by changes in the local magnetic field within their environment (Kirschvink, 1989; Wiltschko and Wiltschko, 1995). More specifically, it has been shown that some of them, including honeybees (Gould, 1980), homing pigeons (Walcott, 1980), salmon (Quinn, 1980) and others (Wiltschko and Wiltschko, 1995), use geomagnetic field information for orientation, homing and foraging. However, the process that animals use to detect the geomagnetic field is unknown (Wiltschko and Wiltschko, 1995). The observation that magnetotactic bacteria use the magnetic properties of biomineralized magnetite nanoparticles (Blakemore, 1975), led to the hypothesis that intracellular biomineralized magnetite could interact with the geomagnetic field monitoring information on its intensity and direction. Identifying the presence of magnetite particles in different organisms, whose behaviour is influenced by the geomagnetic field, is a first step towards demonstrating that biogenic magnetite is involved in geomagnetic field detection.

It has been shown that the ant *Formica rufa* uses information from the geomagnetic field for orientation during the foraging process (Çamlitepe and Stradling, 1995). The same effect was studied in the ant *Solenopsis invicta* (Anderson and Vander Meer, 1993), but there is some discussion about the reproducibility of these results (Klotz et al., 1997). The presence of ferric (FeIII) iron in the abdomens of *Solenopsis invicta* workers was demonstrated by Slowik and Thorvilson (1996), but they failed to show that this was in crystalline form and that it was a magnetic material. Using electron paramagnetic resonance techniques, Esquivel et al. (1999) showed that for this same species of ant a magnetic signal could be related to magnetite nanoparticles.

In the present study, we report on the presence of magnetite particles in the ant *Pachycondyla marginata*. These ants, common in the southeast region of Brazil, present two interesting characteristics: they are termitophageous ants, exclusively preying on live termites (*Neocapritermes opacus*), and they are migratory, changing their nest site from time to time. Leal and Oliveira (1995) followed the migrations of different colonies and, from these results, we infer a preference for the south-north direction, suggesting some influence of the geomagnetic field on the choice of migration route.

Materials and methods

Pachycondyla marginata (Roger) ants were collected in the region of Campinas, São Paulo, Brazil, and preserved in 80 % (v/v) ethanol, after being washed in the same solution. Ants were separated into three parts: head, thorax and abdomen. The isolation procedure consisted of maceration in the presence of 5 % sodium hypochlorite (NaOCl) solution (Reagen, Brazil) with visual separation of cuticle where possible. Approximately equal amounts of each isolated sample were placed in 1.5 ml Eppendorf tubes, and more NaOCl solution was added to half-fill the tubes, which were left overnight at 4 °C. The tubes were centrifuged at 15,700 ***g*** for 8 min to pellet the insoluble material. The supernatant consisting of dissolved organic matter was discarded and a further 0.75 ml of NaOCl solution was added to each pellet. After resuspending the pellets both manually and using ultrasonic vibration for 15 min, the material was subjected to magnetic precipitation by placing a strong magnet on the lower lateral wall (not the bottom) of the tubes for 10 min. The precipitate in the bottom of the tube was removed with a pipette and a final centrifugation was performed to recover the magnetically isolated mineral fraction. As the isolated material contained a considerable amount of fat, washing procedures similar to the one described above were performed by changing the sodium hypochlorite with either chloroform or acetone. This process was repeated until most of the organic matter and fat had been digested and the pellet did not change colour after new cycles of treatment. The insoluble material was washed in distilled water and pelleted by centrifugation as described above. The resulting material was dropped onto an electron microscope grid previously covered with Formvar film (0.5 % (w/v) in chloroform) and observed by transmission electron microscopy (Jeol–100 CX) at 80 kV. Selected area diffraction (SAD) patterns of the isolated particles were obtained using the low diameter aperture (20 μm) of the intermediate lens. The mineral isolates were analysed in a transmission electron microscope Zeiss CEM–902 equipped with an in-column Castaing–Henry spectrometer, in order to obtain electron spectroscopic images. These images are formed by using beam electrons that have lost energy to ionise sample atoms in specific shells. The minimum ionisation energy for each electron in the shell is called edge and is characteristic of the atom. In the present images, the energy loss 532 eV corresponding to the *K* edge was used for the oxygen map, while the energy loss 708 eV corresponding to the $L_{2,3}$ ionisation edge was used for the iron image (for details of the technique, see Reimer et al., 1988). This approach was used to identify iron oxide particles and to obtain a profile of the size distribution of the iron oxides present in our isolated material.

Results

During the process of isolating the magnetic minerals, it was observed that both abdomen and head isolates formed two separate pellets, one in the bottom of the tube and another at the side of the tube in the direction of the magnet, whereas

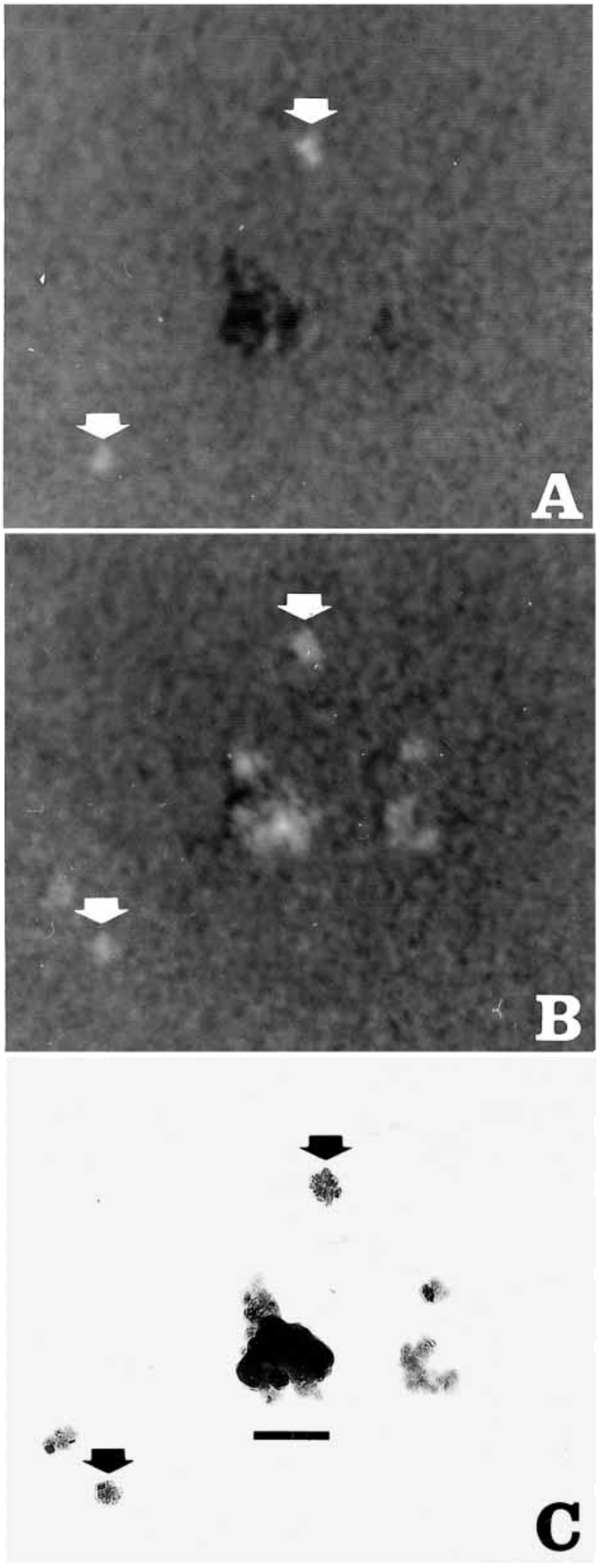

Fig. 1. Electron spectroscopic images of nanoparticles isolated from abdomens. Images were obtained in the energy loss branch corresponding to iron (A) and oxygen (B). The corresponding bright-field image is shown in (C). Scale bar, 200 nm. Arrows indicate the two particles that share iron and oxygen. For iron: maximum energy, 718 eV; minimum energy, 690 eV; slit, 20 eV. For oxygen: maximum energy, 550 eV; minimum energy, 500 eV; slit, 20 eV.

Table 1. *Comparison of the experimental lattice distances (*d*), calculated from the electron diffraction pattern shown in Fig. 3, to the known patterns for different iron oxides*

d (nm)	γ-Fe_2O_3 maghemite	Fe_3O_4 magnetite	α-Fe_2O_3 hematite	FeO wuestite
0.3688	–	–	0.3684 (30 %)	–
0.2593	0.2638	–	–	–
0.2532	0.2517 (100 %)	0.2532 (100 %)	–	–
0.2487	–	–	–	0.249 (80 %)
0.2445	0.2412	0.2424	–	–
0.2300	0.2315	–	0.2292	–
0.2161	–	–	–	0.2153 (100 %)
0.1704	0.1704 (10 %)	0.1714 (10 %)	–	–
0.1585	–	0.1616 (30 %)	0.1599 (10 %)	–
0.1496	0.1476 (34 %)	0.1484 (40 %)	0.1486 (30 %)	0.1523 (60 %)
0.1436	0.1432	–	0.1454 (30 %)	–
0.1412	0.1392	0.1419	0.1414	–

The percentages in parentheses are the relative intensities measured from X-ray powder diffraction, and are directly related to the probability that each diffraction point appears in any diffraction pattern. Lattice distances of other materials containing iron and oxygen, such as the iron aluminium silicates, do not correlate with our experimental *d* values.

X-ray powder diffraction data were obtained from the Joint Committee for Powder Diffraction Standards (JCPDS) database. The corresponding card numbers in this database are: 33-664 for hematite (rhombohedral), 19-629 for magnetite (cubic), 39-1346 for maghemite (cubic), 6-615 for wuestite (cubic).

thorax isolates only formed a precipitate in the bottom of the tube. We analysed the minerals isolated from the head, thorax and abdomen of *Pachycondyla marginata* and obtained electron spectroscopic images of iron and oxygen from the nanoparticles present (Fig. 1). By measuring the size of these particles directly from the electron micrographs, we obtained their size distribution. Since particles are elongated, the distribution of the length (major dimension), the width (minor dimension) and the ratio of these two were plotted. Fig. 2 shows the corresponding histograms for each body segment. While the size distribution of particles in the abdomen corresponds to a single distribution, there is a bimodal splitting of this distribution in thorax and head, suggesting the existence of two size populations of nanoparticles with iron and oxygen. The mean values for length and width of the particles are however, approximately the same in the three body segments: for the abdomen: length, 39±2 nm, width, 26±1 nm; for the thorax: length, 38±3 nm; width, 27±3 nm; for the head: length, 39±2 nm; width, 26±2 nm.

The total number of iron/oxygen-containing particles relative to the total number of electron micrographs obtained for each part of the body determines the relative number of particles analysed in each region of the body of the ant. A relationship of 3:1:2 was found for the relative number of particles in the abdomen, thorax and head, respectively. Fig. 3A shows one example of an SAD pattern obtained from one group of particles from the abdomen (Fig. 3B). Table 1 gives the distances of the diffraction planes obtained from the micrograph in Fig. 3A and shows that they are in agreement with the distances of four different iron oxides. Magnetization measurements (data not shown) were performed using powdered samples of ants and these showed that while ferromagnetic behaviour was present in heads and abdomens it could not be detected in thoraxes. As a consequence SAD patterns were only obtained for material isolated from abdomens and heads.

Discussion

In this paper, we have shown the existence of iron oxides in the body of the ant *Pachycondyla marginata*. Different size distributions were observed in material extracted from the head and the abdomen. The larger amount of nanoparticles found in the abdomen could be related to biomineralization processes and to the accumulation of ingested minerals in the digestive apparatus, while the nanoparticles extracted from the head could be derived from biomineralization processes and/or cuticular contamination. If all the nanoparticles found were from soil contamination, then we should find the same size distribution function in all parts of the body, but this is not the case, reinforcing the idea that biomineralized iron oxide particles are present.

The relative number of particles found in the thorax was low, and no magnetic precipitates visible to naked eye or ferromagnetic behaviour in magnetization measurements were found in this region. We consider, therefore, that the particles found in the thorax could be contamination from the head and/or abdomen, resulting from inexact cuts when the thorax was detached from the other regions of the body.

The diffraction patterns obtained from the particles were always from more than one crystal, which is why Table 1 shows a comparison of the interplanar distances with different iron oxides. The diffraction patterns correspond to mixtures of iron oxides, which may result from the activity of NaOCl, an oxidant, interacting with magnetite to degrade it and produce maghemite, hematite or even other iron oxides (Towe, 1985),

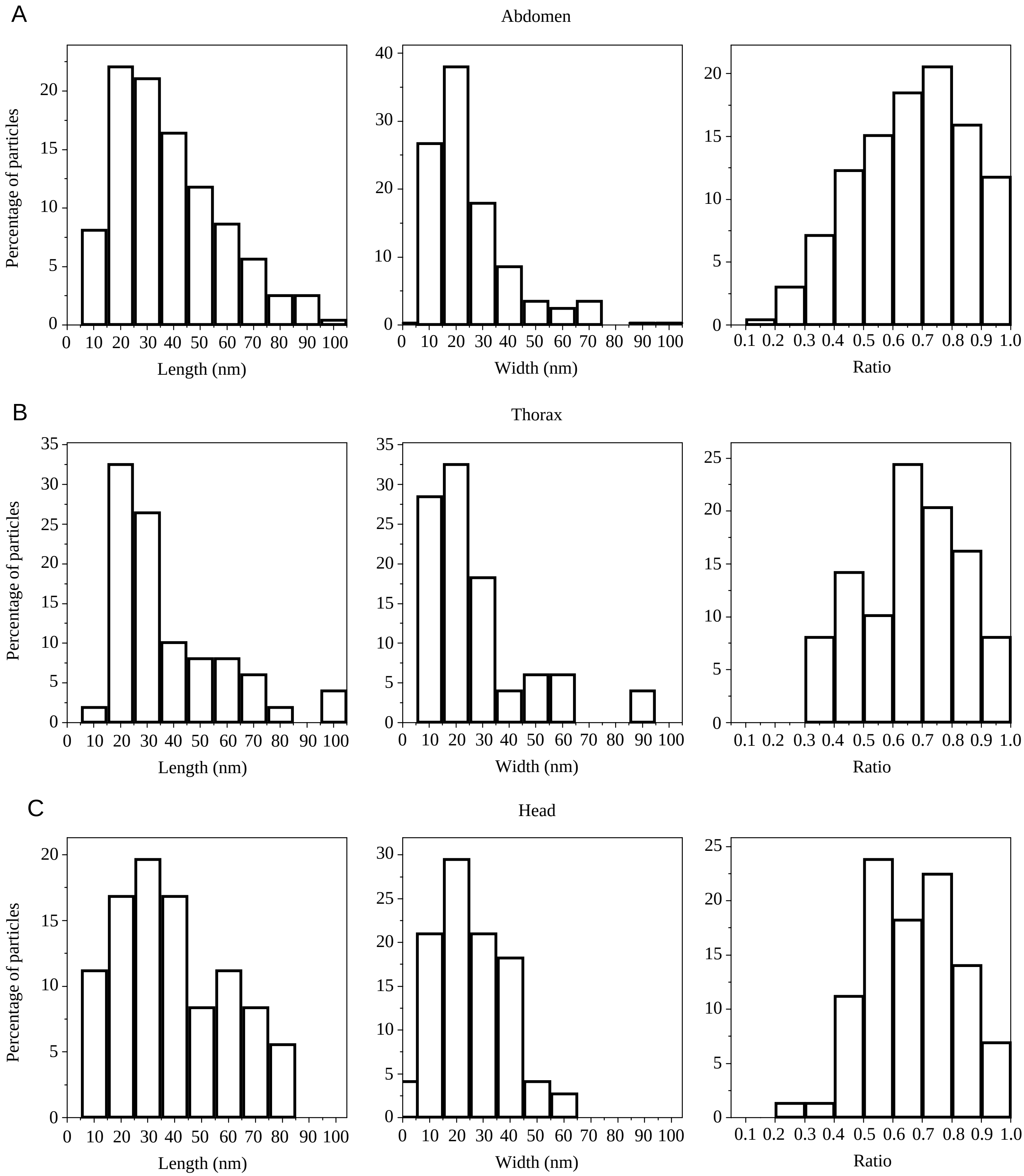

Fig. 2. Size distribution histograms from the particles extracted from the abdomen (A; *N*=194), thorax (B; *N*=49) and head (C; *N*=71) of *Pachycondyla marginata*. *N*, total number of particles used in the distribution; the ratio values are length:width.

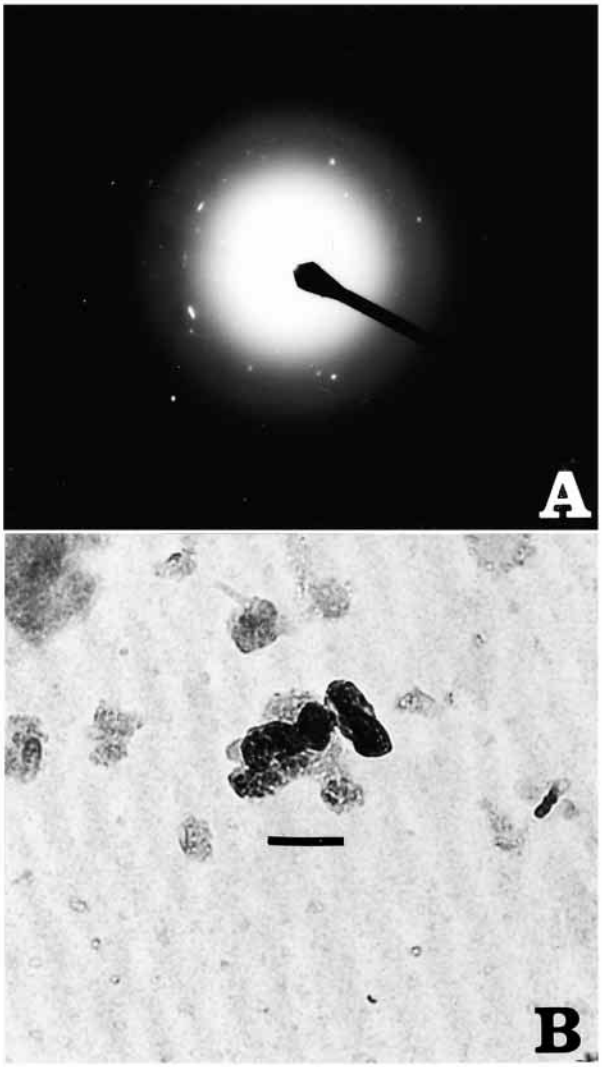

Fig. 3. (A) Selected area diffraction pattern from crystalline nanoparticles isolated from the abdomen. (B) Bright-field of the set of particles that produced the diffraction pattern showed above. Scale bar, 110 nm.

depending on the reaction time. However, most of the diffraction patterns agree most closely with maghemite and we cannot discard the possibility that it is normally present, independent of magnetite degradation.

Both magnetite and maghemite are magnetic iron oxides and are good candidates for the model of geomagnetic field detection based on an interaction between magnetic nanoparticles and the geomagnetic field. To lend further support to this hypothesis, it is still necessary to look for biomineralizing cells, which should be in contact with nervous system terminations to transmit the information on the magnetic field detected.

In conclusion, we have shown the presence of magnetic iron oxides in the head and abdomen of the ant *Pachycondyla marginata*. This makes it possible to explain their migratory behaviour on the basis of magnetic orientation. The volume of magnetic nanoparticles determines the thermal stability of their corresponding magnetic moments. In bulk material, the stability is obtained by the formation of domains that minimizes the energy of the system. As the volume of the particle is below a critical value, the magnetic moment is organized in a single domain. Two regions of stability are determined in this case: particles with fixed and stable magnetic moment orientation (single-domain particles) and particles whose magnetic moment is driven by thermal fluctuations and so are without a defined magnetic moment orientation (superparamagnetic particles). Butler and Banerjee (1975) derived the magnetic stability diagram for retangular parallelepipeds of magnetite at 290 K as a function of the width/length ratio. The theoretical boundaries between two-domain, single-domain and superparamagnetic were given. The linear dimensions of the larger magnetic particles found in the head of the ant fit the single-domain region of this diagram while the dimensions of the small particles indicate that they belong to the superparamagnetic region. We speculate that crystals with different sizes can be related to different functions in magnetic field detection. Although there are increasing numbers of reports on both the effects of magnetic fields on animals and the occurrence of biomineralized magnetic particles in animals, the magnetoreception models remain poorly developed. Honeybees are the most studied insects with respect to magnetic sensitivity and it has been proposed that single-domain magnetite crystals determine their compass sense while superparamagnetic magnetite particles are associated with a learned navigational map sense of magnetic intensity gradients (Gould, 1985). Subsequently, Schiff and Canal (1993) have suggested that these superparamagnetic particles are involved in gradient detection by amplifying local changes in the geomagnetic field. Another possibility is that superparamagnetic particles are responsible for 'sensing' the spatial anisotropy imposed by local variations of the geomagnetic field. This would be an active process involving the continuous detection of local magnetic gradients and their daily variations (Wiltschko and Wiltschko, 1995).

Like honeybees, ants are social insects. Although terrestrial, they make frequent movements of their head and we speculate that the different crystal sizes found in the head may be involved with magnetoreception in a similar way to the particles found in the abdomen of the bee.

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