

Brazilian ants diversity and the local geomagnetic field: a ferromagnetic resonance study

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Received 3 June 2005; accepted 7 September 2005

Key words: ant orientation, magnetic material, ferromagnetic resonance

Abstract

Ants have the ability of homing and some species can migrate or move over long distances (nomadic). The presence of magnetic particles as geomagnetic sensors is the most accepted hypothesis to explain ant orientation mechanisms. The room temperature Ferromagnetic Resonance (FMR) spectra of migratory, nomadic, arboreal, trap-jaw and fire ants, applied to 11 samples are presented. The spectra were studied taking into account two components: the low field (LF) with a maximum at g_{\max} values higher than 8 and the high field (HF) at the $g_{\text{eff}}=2.1$ with a linewidth of about 900 Oe. This study tests the systematization plausibility of ant magnetic material characteristics based on absorption spectra area and the ratios between the peak-to-peak amplitude spectral components (LF/HF). The HF component predominates in the spectra of the migratory and one nomadic ant, while the LF is the dominant one in the arboreal and six fire ants studied. The *Solenopsis* absorption spectra area, proportional to the magnetic material amount, increases as the local magnetic field intensity increases, suggesting an adaptation of these ants to the magnetic environment characteristic.

Introduction

Magnetoreception is a complex orientation mechanism to detect the geomagnetic field. It has gained attention in the last two decades. Focus has been given to the study of bees, pigeons and salmon, although effects of magnetic field and the presence of magnetic particles were observed in a wide variety of animals (Wilstchko and Wilstchko 1995). Ants are one of the most abundant social insects as a result of adaptive capability. A high diversity of species is observed in tropical regions (Fittkau & Klinge 1973; Hölldobler & Wilson 1990). They can be found in all forest strata and comprise more than 50% of the arthropods in the tree crown (Erwin & Strork 1983; Adis et al. 1998;

Stork 1988). Geomagnetic orientation has been successfully investigated in *Formica rufa* (Çamlitepe & Stradling 1995) while in *Solenopsis invicta*, behavioral responses to magnetic stimuli are still inconclusive (compare Anderson & Vander Meer 1993 with Klotz et al. 1997). Migration of the *Pachycondyla marginata* ant, during the dry/cold season, was shown to be significantly oriented 13° with the magnetic North–South axis. The geomagnetic field was then suggested as an orientation cue (Acosta-Avalos et al. 2001).

The ferromagnetic hypothesis, based on magnetic particles as the geomagnetic sensor, is well accepted (Vácha 1997; Fleissner 2003). Different techniques are necessary to detect, identify and characterize the particles, which can provide

information to the development of magnetoreception models. High-resolution electron microscopy and magnetometry have traditionally been used to investigate biomineralized magnetite in organisms. The first one requires purification of the magnetic material, which is found in very low concentrations, and although the second one can be based in a high sensitive SQUID detector, its result is the total sample magnetization that contains information of any magnetic component in the sample.

Ferromagnetic Resonance (FMR) has proved to be an efficient tool for these studies (Esquivel et al. 1999; Wajnberg et al. 2000; El-Jaick et al. 2001; Alves et al. 2004, Abraçado et al. 2005; Oliveira et al. 2005) with the advantage of using whole tissues without particle extraction. FMR spectra of smashed whole and body parts of *Solenopsis* ants and *Pachycondyla marginata* ant and *Apis mellifera* honeybee abdomens were shown to be composed of at least three broad components, two of them associated to ferromagnetic particles, probably magnetite. The high field (HF) component at $g \sim 2$ and linewidth larger than 1000 Oe was related to isolated nanoparticles. The other component, called LF, at high g values was associated to these particle aggregates in *S. substituta* head and *P. marginata*, *A. mellifera* and *S. substituta* abdomens and to bulk-like magnetite particles in *S. substituta* thorax. Superimposed to the HF component, a Manganese envelope (ENV) line 600 Oe in width was attributed to amorphous FeOOH as a magnetite precursor or to ultrafine nanoparticles of magnetite (Wajnberg et al. 2000; El-Jaick et al. 2001). Only the HF component was observed in the *Neocapritermes opacus* termite (Alves et al. 2004).

In this paper FMR was used to study six Brazilian ant genus collected in different regions of the country. Spectra of one migratory, two army nomadic, one arboreal, one trap-jaw and six fire ants species were analyzed. The FMR ratios between the amplitude of the spectra components (LF and HF) and the total absorption spectra areas were obtained for comparative analysis. The goal was to verify the sensitivity of this technique for a systematic analysis of magnetic particles (sensors) characteristics in ants. If confirmed it could be generalized for subsequent research on a biologically synthesized magnetic material in insects.

Materials and methods

Ants, proceeding from the cited areas, were collected by hand with a forceps and extensively washed with 80% (v/v) ethanol solution. They were kept in the refrigerator in this solution. Just before measurement ants were dried for 20 min at 50 °C and smashed. As magnetic content varies from one individual to other as previously observed in insects (Gould et al. 1978; Jungreis 1987; Maher 1998; Esquivel et al. 2004), different numbers of individuals (given below) were used to obtain a mean value. Considering the specimen sizes and the FMR signal intensity, at least, three specimens per sample, when possible, were used. All samples were measured a few days after being prepared. The samples were prepared by packing together some individuals into a FMR quartz tube.

Pachycondyla marginata, a migratory and obligate termite predator ant from Campinas (22s54, 47w03), São Paulo; three individuals.

Eciton sp., a nomadic army ant from Teresópolis (22s24, 42w58), Rio de Janeiro; one individual.

Labidus praedator, another nomadic army ant from Teresópolis (22s24, 42w58), Rio de Janeiro; three individuals.

Azteca chartifex, an arboreal ant from Fortaleza (3s43, 38w32), Ceará; hundred individuals.

Odontomachus haematodus, a trap-jaw and predator ant, from Rio de Janeiro (22s54, 43w13), Rio de Janeiro; three individuals.

Solenopsis are commonly known as fire ants and are easily found in tropical areas, in particular in Brazil. Specimens of this genus were collect at different regions. *Solenopsis substituta* from Fernando de Noronha (3s50, 32w24) and from Natal (5s35, 42w37), Rio Grande do Norte; hundred individuals.

Solenopsis nr. substituta from Salvador (12s58, 38w31), Bahia and from Venda Nova (20s20, 41w08), Espírito Santo; hundred individuals.

Solenopsis virulens from Salvador (12s58, 38w31), hundred individuals.

Solenopsis interrupta from Citrolândia (22s36, 43w01), Rio de Janeiro; fifty individuals.

FMR measurements were performed at room temperature with a commercial X-band EPR spectrometer (Bruker ESP300E) operating at 4 mW, with a 100 kHz modulation field of 2 Oe in

amplitude, resulting the first derivative of the power absorption curve as a function of the magnetic field. When necessary spectra were obtained with several scans.

As spectra depend on the sample position in the cavity, care has been taken to get the highest signal amplitude and best resolution of the spectral components. The areas under the absorption curve (derivative spectra second integration) were calculated using a software developed using the graphic language LabVIEW®, starting at the high field extreme where the baseline is defined.

Results

The room temperature FMR spectra of nomadic, migratory and non-migratory ant species from different Brazilian regions are shown in Figure 1. The obtained spectra are normalized to the same receiver gain, number of scan and number of

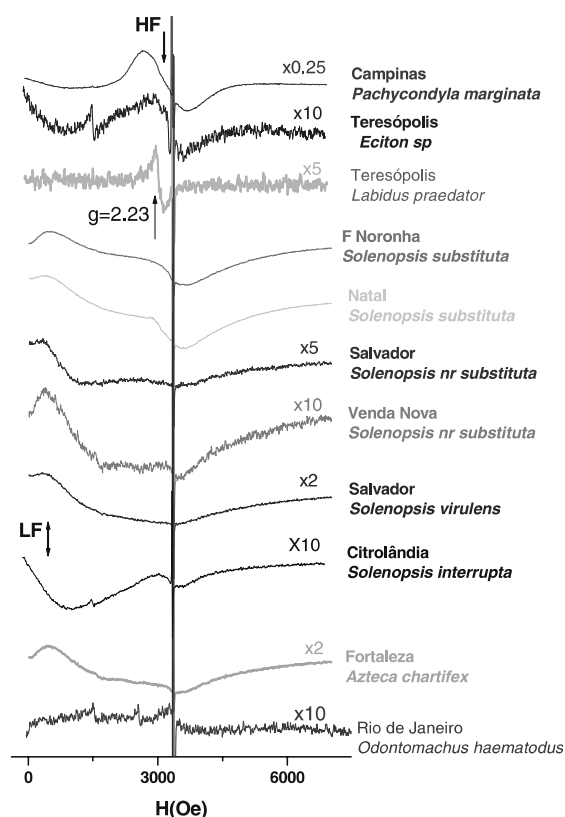


Figure 1. Room temperature FMR spectra of Brazilian ants. Spectra are normalized to the same measurement parameters. Numbers at the end of the spectra are factors used to get a better spectral resolution.

specimens. The numbers near the end of the spectra are extra factors used to give a better resolution to the figure. The narrow line at $g=2$ is related to free radicals usually found in biological samples and it was not considered in this analysis, as well as the weak line at $g=4.3$, characteristic of Fe^{3+} iron in low symmetry environment. The LF and HF spectral components (indicated by arrows in Figure 1) are observed in most of the spectra. These lines were previously established as originating from ferromagnetic particles in ants (Wajnberg et al 2000; Abraçado et al. 2005) and honeybees (El-Jaick et al. 2001), while in termite (Alves et al. 2004) and bacteria (Weiss et al. 2004) only the HF component was observed. The characteristic linewidth and resonant field identify these components. The component at $g\sim 2$ in *S. nr. substituta* (Venda Nova) and *S. interrupta* spectra (Figure 1) are identified as the ENV line by the line width (< 500 Oe). The nomadic *L. praedator* ant spectrum shows a unique line at $g=2.23$ and linewidth of 221 Oe similar to the extra line reported for the honeybee abdomen spectra (El-Jaick et al. 2001). The *O. haematodus* spectrum is of the order of the cavity background with the free radical and Fe^{3+} lines contributions.

The HF line resonant fields, H_{eff} , measured at the field position of zero amplitude fall in the range of 3170–3260 Oe with corresponding $g_{\text{eff}} = hv/\beta H_{\text{eff}}$ varying from 2.12 to 2.07 (*P. marginata* and *A. chartifex*, respectively). The peak-to-peak linewidths, ΔH_{pp} , vary from 710 to 1040 Oe (*Eciton* sp. and *P. marginata* respectively). The LF lines extend to negative field values, so are incomplete in the spectra. As there is a large uncertainty in the H_{eff} determination, the H_{max} position, where the lines amplitude is maximum, are taken with $g_{\text{max}} = hv/\beta H_{\text{max}}$. Except for the *P. marginata*, *Eciton* sp. and *S. interrupta* spectra that H_{max} falls out of the measuring magnetic field range ($H < 19$ Oe), all other g_{max} fall within the 13–21 range (*S. substituta* from Fernando de Noronha and *S. nr. substituta* from Salvador, respectively). The LF amplitude of those three ants spectra was then taken from the first point measured in the spectra and is a low limit value. The g_{eff} , the linewidths and an asymmetry ratio, A (Weiss et al. 2004) were used to classify FMR spectra of biogenic, synthetic and natural inorganic oxides. Among the HF lines observed (Figure 1) only the *P. marginata* and *Eciton* sp. lines are resolved in such a way that a ratio $A\sim 1$ was determined. A ,

g_{eff} and ΔH_{pp} HF values are consistent with superparamagnetic biogenic magnetite particles.

As FMR amplitudes depend on the spectrometer characteristics and gain, it is difficult to determine absolute values of the total magnetic material from FMR spectra, but relative values are reliable. The LF and HF components in the ant spectra were related to magnetic particles of different sizes from temperature dependence analysis (Wajnberg et al. 2000; El-Jaick et al. 2001), so their intensity ratio is used to identify which magnetic size particle is predominant in each ant. The area of the HF FMR absorption curve was shown to be related to the magnetic material amount in termites, by its linear relation to the sample saturation magnetization (Oliveira et al. 2005). Even considering that the linewidth depends on different parameters (Raiker and Stepanov 1992; Berger et al. 2001), it can be fitted, from 100 to 300 K, by (Morais et al. 1987; Wajnberg et al. 2000)

$$\Delta H_{\text{pp}} = (5g\beta Sn/D^3)^* \tanh(\Delta E/2k_{\text{B}}T)$$

where ΔE is the magnetic anisotropy energy barrier height. The pre-factor includes the Bohr magneton (β), the spin associated with each magnetic center inside the nanoparticle (S), the number of magnetic centers per particle (n) and the particle-particle distance in the matrix (D). As ΔE in ant body parts ranges from 3.1 to 7.5e-14 erg (Abraçado et al. 2005) it does not strongly affect the tanh values. The pre-factor is then the most relevant parameter when comparing the absorption spectra area. As D varies inversely to the particle concentration, the HF area relation to the magnetic material concentration is confirmed. A good relation between FMR area and saturation magnetization was also shown for *Solenopsis interrupta* body parts which spectra present a low field component (Abraçado et al. 2005a). As the LF lines are incomplete, the second integration are low limit values even using the software developed to consider a base line correction. Considering a LF symmetric lineshape it is roughly estimated that 50% of absorption area is not observed. This contribution is not relevant for the migratory ant spectra and the spectral area of the fire and arboreal ants are equally reduced. It can then be considered for comparative analysis.

The ratios between the amplitude of the LF and HF and the total absorption area of each ant

spectrum, the geomagnetic field parameters (inclination, declination, and intensity in Oe from National Observatory magnetic data, Vassouras, Rio de Janeiro, Brazil; 1990) and latitude and longitude of the nest local are given in Table 1. It is observed that the HF component predominates in the migratory, *P. marginata*, and in the nomadic ant, *Eciton* sp., spectra (LF/HF *Eciton* sp. absorption area is one order of magnitude lower than those of *P. marginata*, while the nomadic *L. praedator* spectrum is of the order of background signal.

Solenopsis ants present similar lineshapes with a predominant LF line. These ants and Arboreal spectra are characterized by LF/HF amplitude ratios higher or equal to 1. These ratios are around one for *S. substituta* spectra from Natal and Fernando de Noronha where the geomagnetic field inclinations are the same, the declinations are similar and the intensities differ by 500γ ($1\gamma = 10^{-5}$ Oe). *S. nr. substituta* spectra present ratios higher than 7.2 for the samples collected in Salvador and Venda Nova where the geomagnetic fields have similar declinations, but different inclinations (5°) and intensities (800γ). The *S. substituta* from Natal and Fernando de Noronha, and the migratory ants *P. marginata* present the highest magnetic content that is more than 10 times that of the *S. interrupta* from Citrolândia with the lowest content.

The dependence of the spectral parameters, absorption area (Figure 2a) and HF to LF intensity ratio (HF/LF, inverse of the table value) (Figure 2b) with the local geomagnetic field intensity are shown. There is a good correlation for both parameters among the *Solenopsis* species. The magnetic material amount (Figure 2a) increases with increasing geomagnetic intensity, suggesting an adaptation of these organisms to the local environment. A similar relation is observed for the HF/LF (Figure 2b), isolated nanoparticles/aggregates fraction, which also increases with the geomagnetic field intensity.

Discussion

As far as we know, this is the first and preliminary FMR systematic study of ferromagnetic material characteristics in ants. Previous study of six ant

Table 1. FMR parameters of Brazilian ant species and geomagnetic field at nest localization.

Ant species	Sample locality	Habitat/ habit	LF/HF	Absorption area (10^8 a.u.)	Geomagnetic field ^a	Latitude longitude
<i>Pachycondyla marginata</i> (P m)	Campinas	Migratory and predatory ant	0.16±0.02	25±4	I = -31° D = -18° H = 0.235 Oe	22s54, 47w03
<i>Eciton</i> sp.(E sp.)	Teresópolis	Primary and secondary forest /Nomadic ant	0.71±0.04	1.5	I = -32° D = -20° 30' H = 0.238 Oe	22s24, 42w57
<i>Labidus praedator</i> * (L p)	Teresópolis	Primary and secondary forest/nomadic ant			I = -32° D = -20° 30' H = 0.238 Oe	22s24, 42w57
<i>Solenopsis substituta</i> (S s1)	Fernando de Noronha	Secondary forest / fire ant	0.95±0.05	48	I = -16° D = -21° 45' H = 0.268 Oe	3s50, 32w24
<i>Solenopsis substituta</i> (S s2)	Natal	Secondary forest / fire ant	1.29±0.04	55	I = -16° D = -22° 15' H = 0.263 Oe	5s35, 42w37
<i>Solenopsis nr. substituta</i> (S nr s1)	Salvador	Secondary forest / fire ant	8±1	7.6	I = -24° D = -22° 30' H = 0.250 Oe	12s58, 38w30
<i>Solenopsis nr. substituta</i> (S nr s2)	Venda Nova	Secondary forest / fire ant	> > 1	9.5	I = -29° D = -22° H = 0.242 Oe	20s20, 41w08
<i>Solenopsis virulens</i> (S v)	Salvador	Secondary forest / fire ant	> > 1	22	I = -24° D = -22° 30' H = 0.250 Oe	12s58, 38w30
<i>Solenopsis interrupta</i> (S i)	Citrolândia	Secondary forest / fire ant	> > 1	4.2±0.2	I = -32° D = -20° 30' H = 0.238 Oe	22s36, 43w01
<i>Azteca chartifex</i> (A c)	Fortaleza	Secondary forest/Arboreal ant	3.5±0.3	23	I = -8° D = -21° 30' H = 0.266 Oe	3s43, 38w32
<i>Odontomachus Haematodus</i> ** (O h)	Rio de Janeiro	trap-jaw and predatory ant			I = -32° D = -20° 30' H = 0.238Oe	22s54, 43w12

^a National Observatory magnetic data, Brazil. 1990. private communication. * Unusual line at $g=2.23$, no HF and LF observed. ** FMR spectrum of the same order of background.

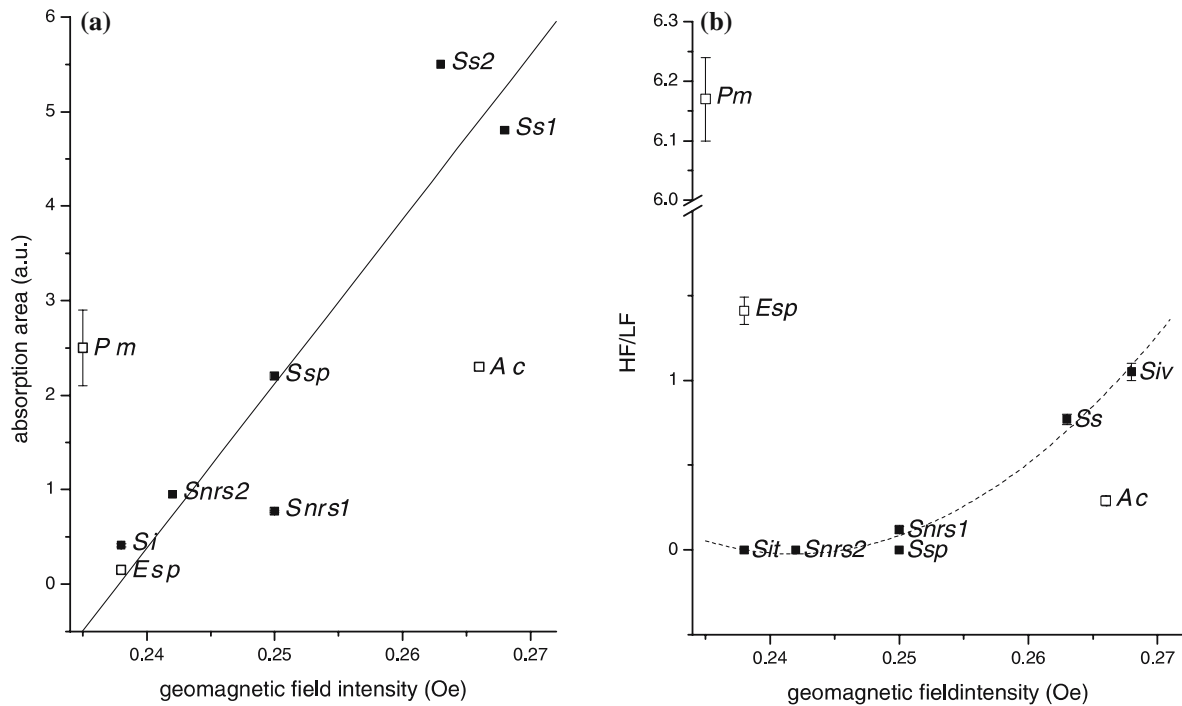


Figure 2. FMR parameters as a function of the local geomagnetic intensity. (a) total absorption spectra area (b) HF/LF intensity ratio. *Pm*, *Pachycondyla marginata*; *E sp.*, *Eciton sp.*; *Ss1*, *Solenopsis substituta* from Fernando de Noronha; *Ss2*, *Solenopsis substituta* from Natal; *Snrs1*, *Solenopsis nr. Substituta* from Salvador; *Snrs2*, *Solenopsis nr. Substituta* from Venda Nova; *Sv*, *Solenopsis virulens*; *Si*, *Solenopsis interrupta*; *Ac*, *Azteca chartifex*.

species has only observed the free radical and Mn lines (Krebs & Benson 1965). Based on the two ferromagnetic components, HF and LF, of insect FMR spectra this study intend to verify whether ant spectra can be identified by the two parameters, intensity ratio and absorption area, and if they are correlated to the geomagnetic field. Spectra were obtained at room temperature because this is the biologically relevant one. A more detail analysis considering component deconvolution, temperature dependence and the use of complementary techniques, would be interesting to add to this study but it is not the scope of this paper. Even though, the results suggest a relation between FMR spectral features and orientation mechanism.

The migratory and the *Eciton sp.* nomadic ant species present more isolated nanoparticles than large ones. *P. marginata* ant presents the highest isolated particle fraction (Figure 2b). The magnetic material amount in *P. marginata* is about ten times higher than in this nomadic ant, while it is FMR undetectable in the *L. preadator* nomadic ant. The low concentration of the ferromagnetic material

relative to the ant body size in the *L. preadator* can be responsible for the difficulty of detecting FMR components. Further measurements using only body parts (increasing the magnetic/biological material ratio) could reveal the magnetic components, if present. Under this point of view, FMR is a promising tool to distinguish migratory from non-migratory ant species, which revisit a place after irregular long periods, suggesting that the predominant isolated and small particles could be involved in the long distance orientation.

Induced Magnetic Remanence (IRM) measurements of nine insects, migrant and non-migrant, that resulted in no strong correlation between magnetic particles and migration habits (Jungreis 1987). In contrast, HF/LF ratio higher than one was shown as a parameter correlated to long distance colonies movements considering the five Formicidae genus studied. The following points can explain the apparent disagreement with the previous result. As it is still unknown how animals, insects in particular, detect the geomagnetic field, low IRM of migratory insect does not necessarily discard the

magnetoreception hypothesis. The highest magnetic remanence was obtained in the mole cricket (*Scapteriscus acletus*) for which migration is not reported but magnetic material could be associated to magnetic orientation in darkness conditions, as reported for mole rats (Marhold et al. 1997). Nevertheless, unexpected relative high quantities of magnetic material were also found in other cricket species for which any suspected behavior requiring a compass mechanism was proposed (Jungreis 1987). Magnetic sensors are expected to be biomineralized with specific characteristics different from synthetic and soil origin ones. These cricket high IRM values could be from ingested magnetic material contributions. Moreover, FMR spectra can be deconvoluted in components associated with different magnetic structures that can be involved in different physiological/biological functions. It is interesting to draw attention to the HF component which is associated to superparamagnetic particles that would not be detected by induced magnetic remanence.

Recently, FMR was proposed as a powerful tool to identify magnetosome chain structures in bacteria and magnetofossil, being sensitive in conditions where the Moskowitz test fails. While most bacteria produce single domain (SD) magnetite chains (Weiss et al. 2004), insect magnetic particles fall mainly in the superparamagnetic (SP) and pseudo single domain (PSD) regions (Acosta-Avalos 1999; Esquivel et al. 2004) with two different structures associated to the LF and HF FMR components. This paper shows that the amplitude ratio of these lines is a promising property to characterize ant moving habits. Interesting to note that the distinguishing ratio ranges point to the relevance of isolated nanoparticles in the orientation mechanism. Both the intensity ratio and the absorption spectra area, associated to the magnetic material amount, are important parameters that correlate well to local geomagnetic field of *Solenopsis* specie nests. The remarkable positive correlation between magnetic material amount and geomagnetic fields deserves further studies considering different orientation mechanism. Other measurements are necessary to verify these relations under a future systematization, yet the soil composition influence cannot be ruled out and deserve further analysis. *Solenopsis* ants are then, adequate to verify the correlation of the magnetic material to the local geomagnetic field, due to the high diversity of easily found species.

Comparative studies on a variety of species, using the same experimental procedure, are relevant to determine whether similarities are based on common magnetoreception mechanism, without the question of different results coming from different experimental details. The relevance of the opposite approach, where one ant specie magnetic material studied by a variety of techniques, should also be considered. The study of magnetic material properties in insects is just beginning, and deserves a lot of work to establish correlations among them. Understanding these correlations and the connections of the magnetic material with the neurophysiological system involve multidisciplinary efforts. This paper introduces a possible method of classifying biomineralized magnetic material, which at this stage, should be considered as a starting point. The reported findings may stimulate new hypothesis for magnetoreception focusing scale factors in orientation.

Acknowledgements

We are thankful to P. Pauferro Jr, L. S. Portela and T. M. Guimarães for some FMR measurements and Dr G. R. Cernicchiaro for the software development. D. M. S. E. and E. W. thank CNPq and FAPERJ for financial support.

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