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ORIGINAL ARTICLE

Magnetic material diversity in Brazilian ants: displacement behaviour and environmental adaptability

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Abstract
How geomagnetic field information is collected and processed by insects for orientation and navigation remains elusive. In social insects, magnetic particles are well accepted as magnetic sensors. Ants have the ability to home and hunt, and some migratory and nomadic species can migrate or move over long distances for which magnetoreception is an important mechanism. It was shown previously that ferromagnetic resonance (FMR) spectral parameters of one migratory and one nomadic ant could be distinguished from Brazilian *Solenopsis* ant species and that these parameters correlate to the local geomagnetic field. The present work focuses on genera engaged in long-distance group raids and emigration collected mainly in the Amazon rainforest. A diversity of specimens of the genus was individually measured by FMR. Cluster analysis of the occurrence of the FMR Low Field component, associated with large or aggregated nanoparticles, and their spectral angular dependence resulted in a phylogenetic dendrogram of the genera of ants, principally from the North Brazilian region. The magnetic material characteristics of ants of the Invertebrate Collection from the Museum Paraense Emilio Goeldi were tested looking for their relation to ant genera and the local geomagnetic field. The observed spectral differences of the magnetic particles suggest that they are related to their capacity for adaptation to their environment and/or to displacement behaviour.

Keywords Magnetic material · Ferromagnetic resonance · Phylogenetic analysis · Raiding · Ant adaptability

Introduction

Magnetoreception is a complex animal orientation mechanism based on the detection of the geomagnetic field parameters: intensity (Int), inclination (Incl) and declination (Decl). At present, there are three hypotheses for the detection of the geomagnetic field: electromagnetic induction for aquatic animals, the ferromagnetic one that assumes magnetic particles as the sensor and the chemical one which proposes biochemical reactions that involve the formation of radical pairs (Lohman 2010). Effects of the geomagnetic field and the presence of magnetic particles have been observed in a wide variety of animals (Wilstchko and Wilstchko 1995; Shaw et al. 2015).

Geomagnetic orientation has been successfully investigated in insects for which the ferromagnetic hypothesis is well accepted. Magnetic measurements indicated the presence of nanoparticles in different ant, bee and termite species (Wajnberg et al. 2010). Migration of *Neoponera marginata* (Roger, 1861) 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). Different iron oxide particles were found inside the tissue of three joints of the *N. marginata* antenna that were suggested as a magnetic sensor, probably incorporated from the soil (Oliveira et al. 2010). More recently, titanium and iron titanium oxide were also reported in these joint sections (Wajnberg et al. 2017). Incorporation was tested by behavioural experiments with *Atta colombica* (Guérin-Ménevele, 1844). It was concluded that *A. colombica* also requires contact with soil to incorporate magnetic particles that can be used as a magnetic compass (Riveros et al. 2014).

Foraging and nest displacement behaviours, that involve orientation mechanisms, vary according to genera and

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species (Lanan 2014). Ants' foraging strategies were classified into two categories: no recruitment and recruitment in group. In both cases they make a trail pheromone on the ground (Billen 1992) which marks all way from the food source to nest. In the first case, one specimen (scout) leaves the nest to search for food, and return alone. In the second one, one scout alone or a group of scouts orients visually to a food resource. When they orient back home to recruit the followers, a pheromone trail is laid down on the ground (Billen 1992) which marks all way from the food source to the nest. This kind of recruitment occurs in many ant genera such as poneroid like *Neoponera* (Emery, 1901) and *Mayaponer*a (Schmidt and Shattuck 2014); Schmidt 2013; Fernandes et al. 2014), in formicoid like *Solenopsis* (Westwood, 1840) (Hölldobler and Wilson 1990), and the dorylomorph army ants, as *Eciton* (Latreille, 1804) and *Labidu*s (Jurine, 1807) (Lanan 2014; Schmidt 2013).

All army ants are predators and nomadic, and may be subterranean or epigaeic (above-ground). Their aggressive foraging behaviour is known as raiding, in which a large numbers of ants feed simultaneously over a certain area, such as in *Eciton* and *Labidus* species. Many kinds of animals such as birds, frogs, lizards, scorpions, spiders, insects and other invertebrates can be found associated with them building a very interesting bioecological relation when they are moving (Willson et al. 2011; O'Donnell et al. 2011; Dejean et al. 2014; Rettenmeyer et al. 2011). Army ants form a bivouac that is a nest constructed with the bodies of living ant workers to protect the queen and immature ants. The sizes of these bivouacs varies from 150.000 individuals in *Eciton burchelli* (Westwood, 1842) to over one million in *Labidus praedator* (Fr. Smith, 1858) and the number is unknown in *Labidus spininodis* (Emery, 1890) (Wilson 1971). *Neoponera commutata* (Schmidt and Shattuck 2014) is specialist predator on termites of the *Syntermes* genus (Wild 2002). It is also a migratory ant that, like *N. marginata* and *N. laevigata* (Smith, 1858) relocates nest sites at irregular intervals (Hölldobler et al. 1996).

Ferromagnetic resonance (FMR) has proved to be an efficient tool to identify large fractions of intact, non-interacting bacterial magnetosome chains. A first study systematically analysed the FMR parameters of dozens of samples of magnetite and other materials to group them and identify their biogenic origin (Weiss et al. 2004). Later, a quantitative analysis of the magnetic anisotropy in magnetosome chains of both cultured and natural magnetotactic bacteria, based on a phenomenological theory, was proposed by fitting of FMR spectra with model calculations (Charilaou 2017 and references therein). Otherwise, in animals the magnetic material is found in very low concentrations, distributed in all body parts, but FMR has shown to be a useful technique to study the variety of magnetic particles in social insects, in particular, in ants (Wajnberg et al. 2010 and references therein).

The diversity of the FMR spectra of Brazilian ants (Wajnberg et al. 2005) was studied for species collected in different regions searching for a correlation of the spectral parameters to the local geomagnetic field components. The spectra of *Sonelopsis* (Westwood, 1840) species, a migratory and an army ant, were studied taking into account the absorption spectra area and the ratio between the intensity of the two spectral components associated with different magnetic particles, the low (LF) and the high (HF) field. Comparative FMR studies on a variety of insect species are relevant to determine whether nanoparticle similarities are associated with a common magnetoreception mechanism. The study of magnetic material properties in insects still calls for a lot of work to establish these correlations.

Magnetoreception can play an important role in ant orientation and navigational behaviours such as running, raiding, homing, colony migration, mainly when other orientation cues are absent. Ants presenting migratory and nomadic behaviours are interesting to search for magnetic nanoparticles as possible sensors for magnetoreception. Nomadic ants lack a permanent nest; instead, colonies alternate between a stationary stage, during which the queen is enlarged and laying eggs, and a nomadic phase, during which the colony often moves long distances in search of food for their voracious larvae. Migratory ants move their nests in steps, that start by some workers actively excavating the new nest, followed by eggs, larvae and pupae transportation by the workers; and ends by relocation of alates, the queen accompanied by many workers. The aim of this paper was to test the use of FMR spectral parameters, namely absorption area, LF and HF intensity ratio and spectral angular dependence, related to displacement behaviours and local geomagnetic field parameters in nine species of five ant genera, mainly from the North Brazilian region. The following hypothesis were tested: (a) the presence of the LF component and the anisotropy character of the FMR spectra are related to displacement behaviours; b) the spectral area and the HF/LF intensity ratio are related to local geomagnetic field parameters.

Materials and methods

Samples

Ants of the Invertebrate Collection from the Museum Paraense Emilio Goeldi were analszed by Ferromagnetic Resonance. It includes 39 individuals that belong to nine species of the five genera: *Mayaponera, Neoponera, Eciton*, *Labidus and Solenopsis*. They are from six federal states in Brazil (Table 1) and were collected from 1974 to 2011. All individuals analysed are workers, except for three soldiers, two of the *E. hamatum* (Emery, 1894) and one of *E. burchelli* species (a in Table 1). The specimens were collected on the

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Table 1 Specimen identification, collecting details and spectral anisotropy: Aniso pos: anisotropic in position, Aniso int: anisotropic in intensity and Iso: isotropic

Label	Collection number	Specie	Collect place	Collect date	Latitude/longitude	Incl (°)	Spectral character
Eb 1	11,513,796	*Eciton burchelli*	Lago Verde (MA)	04/18/81	03°57′25″S/44°49′19″O	2.41889	Aniso pos
Eb2	11,513,840	*Eciton burchelli*[a]	Ji-Paraná (RO)	08/12/84	10°53′07″S/61°57′06″O	2.86889	Iso
Eb3	11,516,818	*Eciton burchelli*	Parauapebas (PA)	07/03/85	06°04′04″S/49°54′07″O	2.49306	Iso
Eh4	11,513,885	*Eciton hamatum*	Ourém (PA)	10/27/90	01°33′07″S/47°06′52″O	5.80694	Aniso pos
Eh5	11,513,881	*Eciton hamatum*[a]	Ji-Paraná (RO)	08/10/84	10°53′07″S/61°57′06″O	2.86972	Anisot int
Eh6	11,513,846	*Eciton hamatum*[a]	Ji-Paraná (RO)	08/12/84	10°53′07″S/61°57′06″O	2.86889	Aniso int
Lc1	11,513,992	*Labidus coecus*[c]	Conceição do Araguaia (PA)	11/17–21/1979	08°15′28″S/49°15′53″O	−0.12444	Iso
Lc1a	11,513,992	*Labidus coecus*[c]	Conceição do Araguaia (PA)	11/17–21/1979	08°15′28″S/49°15′53″O	−0.12444	No signal
Lc2	11,514,004	*Labidus coecus*	Bujarú (PA)	04/20/74	01°30′54″S/48°02′41″O	12.13750	No signal
Lc3	11,514,115	*Labidus coecus*[c]	Lago dos Rodrigues (MA)	05/25/79	04°36′32″S/44°58′48″O	2.12833	Not obs
Lc3a	11,514,115	*Labidus coecus*[c]	Lago dos Rodrigues (MA)	05/25/79	04°36′32″S/44°58′48″O	2.12833	Iso
Lc4	11,514,018	*Labidus coecus*	Belém (PA)	04/18/97	01°27′21″S/48°30′14″O	5.23028	Iso
Lp5	11,514,046	*Labidus praedator*	Brasília (DF)	05/04/79	15°47′38″S/47°52′58″O	−14.0806	Iso
Lp6	11,514,001	*Labidus praedator*[c]	Conceição do Araguaia (PA)	11/17–21/1979	08°15′28″S/49°15′53″O	−0.12444	Iso
Lp6a	11,514,001	*Labidus praedator*[c]	Conceição do Araguaia (PA)	11/17–21/1979	08°15′28″S/49°15′53″O	−0.12444	Iso
Lp7	11,514,119	*Labidus praedator*	Lago dos Rodrigues (MA)	05/24/79	04°36′32″S/44°58′48″O	2.12833	Iso
Ls8	11,514,146	*Labidus spininodis*[b]	Melgaço (PA)	11/11/96	01°48′16″S/50°42′44″O	7.14472	Iso
Ls8a	11,514,146	*Labidus spininodis*[b]	Melgaço (PA)	11/11/96	01°48′16″S/50°42′44″O	7.14472	Iso
Ls9	11,514,135	*Labidus spininodis*	Oiapoque (AP)	05/03/79	03°50′35″N/51°50′07″O	22.95778	Iso
Nc1	11,515,680	*Neoponera commutata*	Santa Bárbara do Pará (PA)	06/16/88	01°13′25″S/48°17′40″O	8.44472	Iso
Nc2	11,515,683	*Neoponera commutata*	Serra do Navio (AP)	01/07/80	00°53′44′′S/52°00′08′′O	16.643	Aniso pos
Nc3	11,515,681	*Neoponera commutata*	Breves (PA)	11/18–12/5/1987	01°40′56″S/50°28′49″O	10.02500	Aniso pos
Nc4	11,515,678	*Neoponera commutata*	Terra indígena Alto Turiaçu (MA)	07/18/81	02°22′08″S/4600′07″O	6.39472	Aniso int
Nc5	11,506,392	*Neoponera commutata*	Parauapebas (PA)	6/17–20/1985	06°04′04″S/49°54′07″O	2.50722	Aniso pos
Nc6	11,515,679	*Neoponera commutata*	Ouro Preto do Oeste (RO)	9/4/1985	10°44′53″S/62°12′57″O	3.115	Aniso pos
Nc7	11,515,682	*Neoponera commutata*	Melgaço (PA)	02/24/03	01°48′16″S/50°42′44″O	5.08806	Iso
Mc1	11,515,684	*Mayaponera constricta*	Melgaço (PA)	10/29–31/2003	01°48′16″S/50°42′44″O	4.85861	Iso
Mc2	11,515,685	*Mayaponera constricta*	São Francisco do Pará (PA)	06/02/79	01°10′10″S/47°47′43″O	11.03166	Iso
Mc3	11,515,687	*Mayaponera constricta*	Novo Aripuanã (AM)	09/07/11	05°08′00″S/60°22′30″O	5.79444	Iso
Mc4	11,515,688	*Mayaponera constricta*	Humaitá (AM)	08/16/11b	07°30′22″S/63°01′15″O	3.63639	Aniso pos

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Table 1 (continued)

Label	Collection number	Specie	Collect place	Collect date	Latitude/longitude	Incl (°)	Spectral character
Mc5	11,515,689	*Mayaponera constricta*	Humaitá (AM)	08/19/11	07°30′22″S/63°01′15″O	3.63639	Anisotint
Mc6	11,515,686	*Mayaponera constricta*	Belém (PA)	02/06/79	01°27′21″S/48°30′14″O	11.29361	Aniso pos
Sb1	11,515,692	*Solenopsis bruesi*	Belém (PA)	02/21/08	01°27′21″S/48°30′14″O	1.40722	Aniso pos
Sb2	11,515,693	*Solenopsis bruesi*	Belém (PA)	02/21/08	01°27′21″S/48°30′14″O	1.40722	Aniso pos
Sb3	11,515,694	*Solenopsis bruesi*	Belém (PA)	Gm02/21/08	01°27′21″S/48°30′14″O	1.40722	Iso
Sb4	11,515,695	*Solenopsis bruesi*	Belém (PA)	02/21/08	01°27′21″S/48°30′14″O	1.40722	Iso
Sg5	11,515,690	*Solenopsis gayi*[b]	Belém (PA)	02/24/08	01°27′21″S/48°30′14″O	1.40444	Iso
Sg5a	11,515,690	*Solenopsis gayi*[b]	Belém (PA)	02/24/08	01°27′21″S/48°30′14″O	1.40444	Iso

[a]Soldiers. All other individuals are workers

[b]Double collected specimen of the same nest

[c]Double collected specimen considered belonging to the same nest

ground surface of primary and secondary forests at lowland and high land areas by using sardine baits, pitfall traps and manual techniques. All specimens were first preserved in 75% alcohol and then sorted, dried, glued in paper triangles, pointed in pins, organized by species and conserved in small boxes, in entomological cabinet draws, at room temperature of 20–22 °C. *S. gayi* and *L. spininodis* were collected at the same date and city and are of the same nest (b in Table 1). Other specimens, marked with c in Table 1, were sampled together, kept in the same pin and were considered as from the same nest.

FMR

FMR measurements were performed at room temperature with a commercial X-band EPR spectrometer (Bruker ESP300E) operating at 16 mW, with a 100-kHz modulation field of 4 Oe in amplitude, 8 scans and 6.3e4 receiver gain, resulting in the first derivative of the power absorption curve as a function of the magnetic field. When necessary, spectra were obtained with 4 or 16 scans.

The FMR principle is similar to that of Electron paramagnetic resonance (EPR) and relies on Zeeman splitting. While EPR deals with isolated atoms, FMR basically deals with atoms which are coupled together by exchange interactions. The sample magnetization precesses around the direction of a static magnetic field, at the Larmor frequency. Microwaves of fixed frequency are then applied to the sample, with their energy absorbed depending on the strength of the applied and any internal magnetic fields.

From the resonant condition, the effective spectroscopic splitting factor g_{eff} is $h\nu/\mu_B H$, where h is the Planck constant, ν the microwave frequency, μ_B is the Bohr magneton and H the zero-crossing magnetic field position of a spectral component or the applied magnetic field related to a spectral feature. The peak-to-peak linewidth, ΔH, is the magnetic field difference between the points where the amplitude of the derivative of the absorption are maximum and minimum and the line intensity is the difference of amplitude at these points. These parameters were obtained using the Winespec software, specially developed for manipulation of a set of EPR data.

The spectra are composed of the high field (HF) and low field (LF) components as previously defined (Wajnberg et al. 2000) which are indicated in Fig. 1. The HF are broad components ($\Delta H > 300$ Oe) in the $g_{\text{eff}} = 2$ region. The LF was arbitrarily chosen as broad components with $g_{\text{eff}} > 4.3$ (Riveros et al. 2014). While the HF line must be due to single magnetic nanoparticles, the LF line must be due to large nanoparticles or aggregates of nanoparticles. The fact that the temperature dependence of the resonance field of these lines generated curves almost parallel to one another led us to correlate them to the isolated and the aggregate magnetic structures, with the magnetic field shift associated with the demagnetizing field contribution(shape anisotropy) (Wajnberg et al. 2000). The LF and HF intensities (difference between the maximum and minimum peaks) were estimated from visual determination of the peak positions. The LF intensity of the incomplete components was taken from the first point measured in the spectra and is a low limit value, hereafter the LF/HF intensity ratio low limit. The absorption area of the spectra was calculated as the second integral of the derivative spectrum starting at high field values where the baseline is better defined, with custom software developed using the graphic language LabVIEW.

As spectra depend on the vertical sample position in the cavity, care has been taken to get the highest signal amplitude and best resolution of the spectral components for each specimen.

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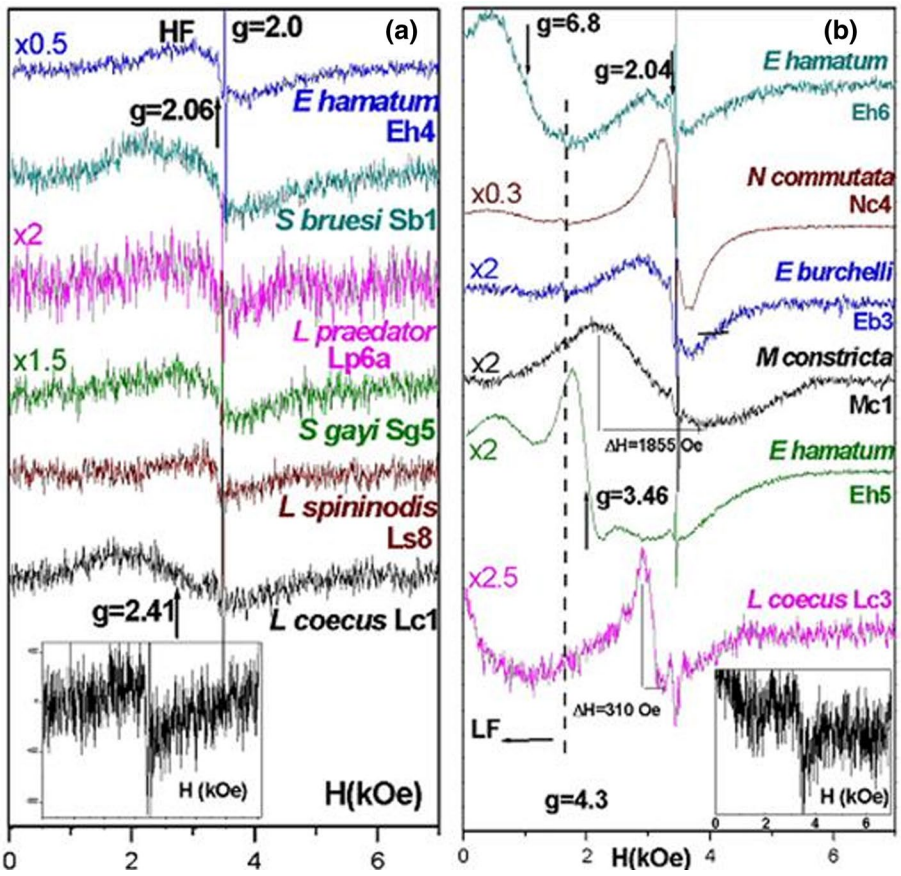

Fig. 1 FMR spectra at room temperature of some specimens presented in Table 1. **a** spectra with lower signal to noise ratio than spectra in **b**. Inserts: spectrum of *L. coecus* specimen of the same nest of that in the Figure. See "Materials and methods" for FMR settings. Vertical scales are in arbitrary units. The scale in **b** is four times that of **a**. Numbers close to the spectra are amplifying factors after normalized spectra to eight scans

The angular dependence of the spectra of the ants was performed 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 (Fig. 2 inset).

When available, geomagnetic data were obtained from the National Observatory in Tatuoca, Pará, Brazil, for the collecting data and place. The International Geomagnetic

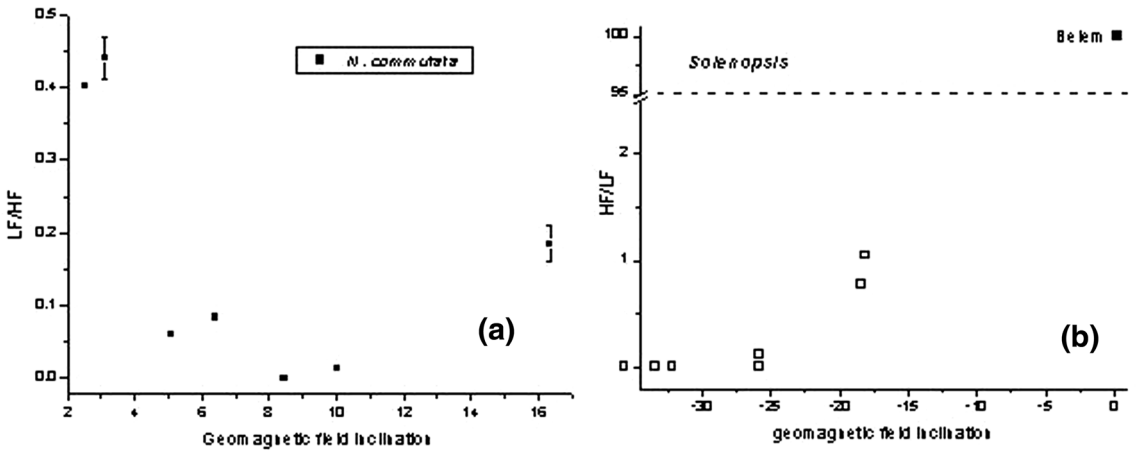

Fig. 2 LF and HF intensity ratios as a function of geomagnetic field inclination, Incl (**a**) LF/HF intensity ratio of *N. commutata* specimens (**b**) HF/LF intensity ratio of *Solenopsis* specimens. Open squares data modified from Wajnberg et al. (2005), horizontal axis: Incl of collecting date and place instead of geomagnetic field total intensity; full square specimens from Belem, vertical position just for visual effect (see text)

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Reference Field (IGRF-12, 2014) model of the Earth's main magnetic field, available at http://www.ngdc.noaa.gov/IAGA/vmod/igrf.html, was also used to obtain the local geomagnetic field parameters of all cities on the, respectively, different collecting dates.

Statistical analysis

The Jaccard or similarity index was used to generate a genera dendrogram that compares the similarity of the spectra of each ant specimen. The isotropic or anisotropic spectral characteristic (Table 1) and the presence of the low field (LF) and high field (HF) components were considered to obtain the similarity indexes. The similarity index J_{AB} among the genera A and B was obtained as folows:

$$J_{AB} = [A \cap B]/[A \cup B], \quad (1)$$

where $[A \cap B]$ is the number of spectral characteristics shared by both genera and $[A \cup B]$ is the total number of characteristics. The dissimilarity index J'_{AB} was calculated as

$$J'_{AB} = 1 - J_{AB}. \quad (2)$$

A dendrogram was constructed based on the J'_{AB} values and using the unweighted pair group method with arithmetic mean (UPGMA) cluster analysis (Choudhuri 2014).

Results

FMR

FMR of 37 of the 39 collected specimens resulted in complex spectra characteristic of ferro(I)magnetic material. It was shown that ants from all nine tested species have magnetic particles with different spectral properties. Only two specimens of *Labidus coecus* [from Conceição do Araguaia (PA) and Bujaru (PA)] spectra, out of six, are of the order of the cavity background. Examples of room temperature FMR spectra of the nine species distributed in five genera are shown in Fig. 1. The HF and LF components (as defined in "Materials and methods"), associated with isolated nanoparticles and to large/aggregated particles, respectively, typical of magnetic material in social insects (Wajnberg et al. 2010 and ref therein) are present in the spectra. Other narrow lines are superimposed to the HF and LF broad components. In some spectra a narrow line ($\Delta H \sim 110$ Oe) at $g_{eff} \sim 2.06$ is resolved. In a few others a very weak line at $g_{eff} = 4.3$ is observed, associated with a Fe^{3+} ion in allow symmetry environment. The narrow line at $g_{eff} = 2$ is related to free radicals usually present in biological samples.

The g_{eff} values of the HF line are in the range from 2.04 (Eh6, Eb2) to 3.46 (Eh5), all from Ji-Paraná (RO), and the linewidth varies from 310 Oe (Lc3 from Lago dos Rodrigues (MA)) to 1855 Oe [Mc1 from Melgaço (PA), Fig. 1b]. These values are consistent with FMR parameters of superparamagnetic particles obtained in social insects (Wajnberg et al. 2010).

As commonly observed in social insects, most of the LF components of the X-band FMR spectra (Wajnberg et al. 2010) extend to negative field values, so are incomplete components which result in a large uncertainty or even make g_{eff} determination impossible. All g_{eff} values are higher than 6, with this minimum value observed in the Eh5 ant spectra (Fig. 1b) from Ji-Paraná (RO).

Differences in the spectral intensity, in the line position, linewidth and in the HF/LF intensity ratio are observed among the genera (Fig. 1). Even among the same species these differences are easily observed, as with for example, *L. coecus* (Lc1 in Fig. 1a and Lc3 in Fig. 1b) and also *E. hamatum* (Eh4 in Fig. 1a, Eh5 and Eh6 in Fig. 1b). It is interesting to highlight that individuals of the same nest present different spectra, such as *L. coecus* from Conceição do Araguaia (PA) Lc1 and Lc1a (Fig. 1a and insert, respectively) and from Lago dos Rodrigues (MA) Lc3 and Lc3a (Fig. 1b and insert, respectively). The intensities of the spectra in Fig. 1b are at least 2 times higher than those in Fig. 1a (scales of vertical axis in arbitrary units).

Besides the locality (local environment conditions and geomagnetic field parameters), other factors can be related to the magnetic particle properties and then to the spectral characteristic. The time interval between collecting and measurement, and the preservation methods can affect the biological tissue and magnetic material of each ant specimen. Ant colonies are capable of adapting to a system that changes over time (Schroeder et al. 2018). As the geomagnetic field changes on time scales of about a year or more (secular variation),it is possible that the magnetic material adaptively responds to this change, so that the collecting date is an important factor.

The following analysis compares the spectral characteristics of individuals collected in the same city and very close dates (at most 3 days of difference). First, spectra of specimens from the same nest were compared, then those of the same species from different nests, and finally those of individuals of the same genera. Individual spectra of the same species from different cities are also compared.

Labidus coecus (Lc1, Lc1a) from Conceição do Araguaia, (Lc3, Lc3a) from Lago dos Rodrigues, *L. praedator* (Lp6, Lp6a) from Conceição do Araguaia, *L. spininodis* (Ls8, Ls8a) from Melgaço and *S. gayi* (Sg5, Sg5a) from Belem were double collected specimen from the same nest. Spectra of double collected individuals of *S. gayi, L. praedator* and *L. spininodis* are indistinguishable within the spectral noise (figure not shown). Nevertheless, the spectra of *L. coecus* (Lc3, Lc3a) showed differences (Fig. 1b and insert): one has

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an extra line at $g_{eff} = 2.28$ ($\Delta H = 280$ Oe) and the intensity of the LF and HF components are about three times higher than those in the other individual spectra, but with very similar field position and linewidth. Only Lc1 from Conceição do Araguaia exhibits a FMR spectruml; Lc1a spectrum is indistinguishable from the cavity background. These differences can be related to differences in the ant nest function-age and evidence the complexity of the nest organization dynamic of each species studied.

M. constrictra (Mc4, Mc5) collected in Humaitá present very similar spectra. In this case, they probably had the same function in the nest even though they are from different localities, which probably means that the spectra are similar in the lowland area along the Madeira River, an Amazon tributary. However, *E. hamatum* soldiers (Eh5, Eh6) from Ji-Paraná collected with 2 days interval, show different spectra. A HF component at $g_{eff} = 3.5$ ($\Delta H = 480$ Oe) is present only in the spectrum of ant Eh5. The LF components are similar but with higher intensity in the Eh6 spectrum. The HF component in the $g = 2$ region comes out with very low intensity relative to the LF one in both spectra. This difference could be due to its environment since the Ji-Paraná specimens came from highland forests.

The spectra of *L. coecus* and *L. praedator* individuals (Lc3 and Lp7) collected in Lago dos Rodrigues show similar LF and HF with different relative fractions. The extra HF narrow ($\Delta H = 290$ Oe) components present in both spectra are at different g values. The LF component observed in the *E. hamatum* (Eh5, Eh6) spectra is not detectable in the *E. burchelli (*Eb2) spectrum, all three soldiers from Ji-Paraná. This *E. burchelli* spectrum presents the HF component in the $g = 2$ region but with intensity about three and two times lower than *E. hamatum* Eh6 and Eh5 spectra, respectively. This result indicates that the spectra vary for each *Eciton* species.

The spectrum of *L. coecus* (Lc1), collected at a geomagnetic field inclination (Incl) of −0.12, does not exhibit the LF component and the HF component is broader than that present in the spectra of the ant Lc3 collected at an Incl of 2.13. Similarly, differences were observed between the spectra of the *M. constricta*, Mc6 and Mc2, collected at Incl 11.3 and 11.03, respectively. Although the HF components are very similar in this species' spectra, the LF component is only observed in the Mc2 spectrum and the extra narrow component at $g_{eff} = 2.26$ ($\Delta H = 150 \pm 50$) only in the Mc6 spectrum. These specimens were collected on different days during 1979, so the spectral differences for the same species of these two genera could be related to the effects of environmental conditions on the magnetic particle system such as geomagnetic field inclination and/or soil composition.

Finally, only three specimens were collected in mining areas: Nc2 from Serra do Navio; and Nc5 (Fig. 3b) and Eb3 (Fig. 1b) from Parauapebas. The spectra of both *N. commutata* are anisotropic and present the same components, while the *E. burchelli* spectrum is isotropic (with anisotropy as defined in the next section), distinct from the two others.

The LF and HF components in the ants' spectra were related to magnetic particles of different sizes and shapes from temperature dependence analysis (Wajnberg et al. 2000; El-Jaick et al. 2001), and their intensity ratio was used to identify which magnetic size particle is predominant in each Brazilian ant species (Wajnberg et al. 2005). In the present paper, the dependence of the values of that ratio on the geomagnetic field parameters of the collecting date and city, Incl and Int, obtained from the IGRF model, were analysed. No clear correlation was observed except for a qualitative tendency of decreasing the LF/HF ratio in the spectra of *N. commutata* with increasing Incl (Fig. 2a) and Int (Figure not shown). This analysis was not possible for the *Solenopsis* genera since there were few individuals per species and they were all collected on very close dates, at the same geomagnetic and environmental conditions (Belem). Nevertheless, it is Interesting to observe that their LF component contribution to their spectra is negligible, so that the HF/LF ratio is very high (Fig. 2b). This result is in good agreement with the correlation for the species previously studied (Wajnberg et al. 2005).

Angular dependence of FMR spectra

The angular dependence of FMR spectra of 35 ants was classified into three groups: anisotropic in line position (Fig. 3a, b) for which the peak positions are dependent on the rotation angle; anisotropic in intensity (Fig. 3c), for which the peak positions (indicated by the vertical lines) are independent of the rotation angle, whereas peak intensities vary; and isotropic (Figure not shown) for which peak positions and peak intensities are independent of the rotation angle. The anisotropic character is related to the magnetic material arrangement and orientation.

In Fig. 3 anisotropic angular dependence of Nc3, Nc5 and Eh5 FMR spectra is shown as examples. The anisotropic character of all ant spectra is given in Table 1.

The HF component anisotropy in position is easily observed in Fig. 3a, with g values from 3.84 at 45° and 180° to 1.52 at 135°. Less notable is the positional anisotropy of the HF component as shown in Fig. 3b. Solid vertical lines in Fig. 3b indicate extreme g values of the HF component that varies from $g = 2.2$ at 180° to 2.4 at 90°. The dashed vertical line indicates the LF component that is anisotropic in intensity. The Eh5 ant spectra in Fig. 3c exhibit anisotropy in intensity. The LF maximum peak at $g_{eff} = 14$ and the HF one at $g_{eff} = 3.46$ (vertical lines) show the highest intensity at 90° and the lowest one at 0°.

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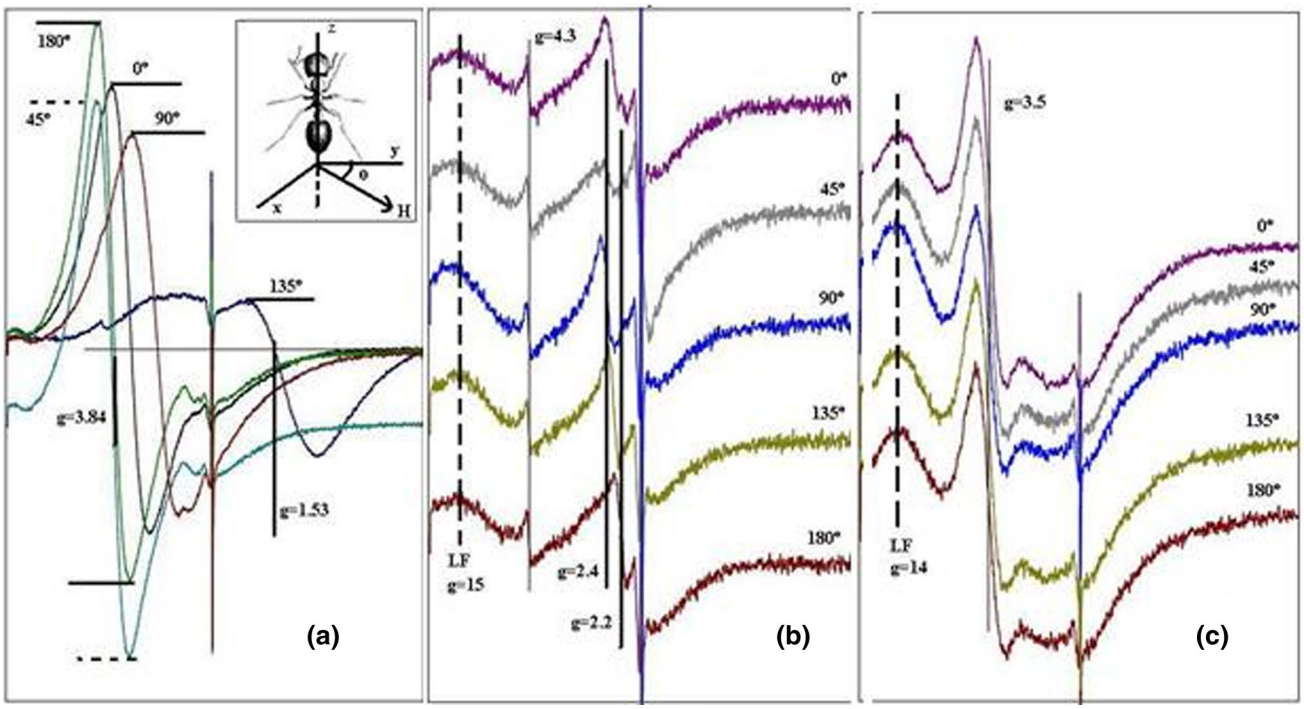

Fig. 3 Examples of angular dependence of FMR spectra. **a** Nc3 ant spectra: anisotropic in line position and intensity, **b** Nc5 ant spectra: anisotropic in line position, **c** Eh5 ant spectra: anisotropic in line intensity. Vertical axes are in arbitrary units. Ant's long body axis positioned in the vertical of the laboratory (z axis) perpendicular to the spectrometer's magnetic field (**H**) rotation plane (xy). Angle $\theta = 0$ was taken for the magnetic field parallel to the ant frontal plane (y axis), in insert. The ant was rotated around its long body axis

About 60% of all ant spectra are isotropic. This percentage varies among the genera. About 70% of the *Solenopsis* and 100% of the *Labidus* spectra are isotropic. Only one of the three spectra of *Eciton* soldiers and workers presents isotropic angular dependence. Workers present spectra with anisotropy in position while the soldier spectra are anisotropic in intensity. This anisotropic character difference could be related to their nest functions, which because of different orientation demand could require different properties of the magnetic material. About 70% of the migratory *N. commutata* spectra are anisotropic. Only about 50% of the *M. constricta* spectra are anisotropic, for which species neither migratory nor nomadic behaviour was reported. This result could be indicative of an anisotropic character related to migration.

Statistical analysis

For similarity tests, the presence of the LF component and the anisotropic spectral characteristics were used. Differences in anisotropy position and in intensity were not considered for this analysis. The HF component was not taken into account as it was present in all the spectra. The four types of spectra (A, B, C and D) were named as shown in Table 2.

Table 2 Definition of the spectral type by its anisotropic character and the presence of LF component

Type	LF	Isotropy	Anisotropy
A	+	+	−
B	+	−	+
C	−	+	−
D	−	−	+

Table 3 Spectral type distribution

Genera	A	B	C	D	Total
Mayaponera	3	0	0	3	6
Neoponera	1	5	1	0	7
Eciton	1	3	1	1	6
Labidus	3	0	7	0	10
Solenopsis	0	0	5	2	7

The distribution of these spectral types for each genus is given in Table 3.

Using the distribution of spectral types shown in Table 3, Jaccard indexes were calculated using Eqs. (1)

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Table 4 Jaccard similarity J and dissimilarity J' indexes among the ant genera

	Mayaponera	*Neoponera*	*Eciton*	*Labidus*	*Solenopsis*
Mayaponera	–	0.307	0.667	0.375	0.384
Neoponera	0.693	–	0.923	0.706	0.428
Eciton	0.333	0.077	–	0.75	0.692
Labidus	0.625	0.294	0.25	–	0.706
Solenopsis	0.616	0.572	0.308	0.294	–

J values are in the table right side (above the diagonal line) and J' are in the left side (below the diagonal line)

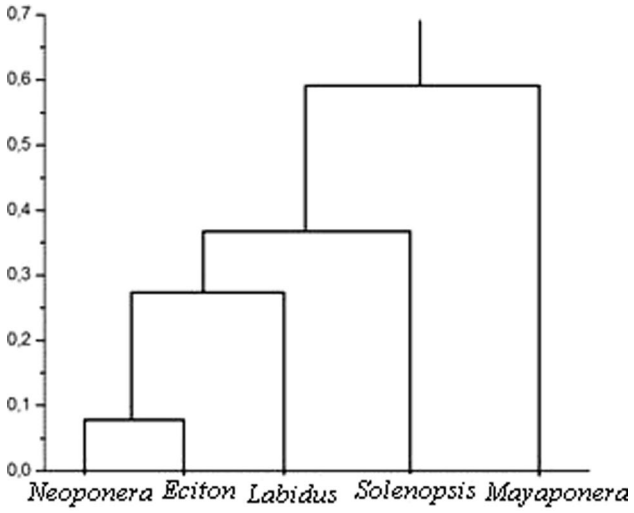

Fig. 4 Dendrogram obtained by the UPGMA cluster analysis using the dissimilarity index J' (Table 4)

and (2). The Jaccard indexes of similarity (J) and dissimilarity (J') are shown in Table 4.

The UPGMA cluster analysis was performed using the J' values (Table 4) and the result is shown as a dendrogram (Fig. 4). The height of the vertical lines of this dendrogram indicates the degree of difference between branches.

The dendrogram shows that *Mayaponera* spectral characteristics are substantially different from the other ant genera. It is also evident that *Neoponera–Eciton* is the only pair of genera that is fairly close, that is, *Neoponera* and *Eciton* genera are more similar to each other than they are to any other one, but still close to *Labidus* genera. *Solenopsis* is closer to the migratory and army ants than *Mayaponera* ants.

Discussion

Nine species of the ant genera *Mayaponera*, *Neoponera, Eciton, Labidus* and *Solenopsis,* mainly from the northern Brazilian region, were studied by FMR looking for the presence of magnetic particles. These species cover a variety of displacement and feeding behaviours, such as, migratory, nomadic, hunt, raid and termitophageous. The specimens analysed are part of the invertebrate collection from the Museum Paraense Emilio Goeldi. The workers of this set were not classified by their functions in the nest as nursers, foragers and scouts and the desirable higher number of individuals of the same nest was not possible.

The present analysis is complex since several parameters, such as conservation time, nest function, specimen processing and the local environment in which samples were collected can influence the spectral features. Differences in ages and functions affect the magnetic material amount and orientation in ants and honeybees (Wajnberg et al. 2010). For example, spectral differences were already reported among *Solenopsis* workers who were nurses, soldiers and foragers collected in the same nest (Abraçado et al. 2009). Even when only foragers of just one nest of the singular *A. colombica* species were collected, the FMR spectra diversity was still observed associated with the variability among individuals with the same nest function as well as the same environmental conditions (Riveros et al. 2014). In the present study, nest function and environmental conditions are neither known nor controlled.

The effect of the conservation condition due to the elapsed time between collecting (older 1979 and newer 2011) and measurement days cannot be neglected. It is then not surprising that most of the *Labidus* spectra are noisy as the specimens were collected in1979. It is interesting to note that two individuals (Lc3 in Fig. 1b and Lp7 spectrum not shown) of different species but collected at the same place and Lc3 1 day after Lp7, presented the same spectral components but with different fractions. It suggests that the components are related to the environmental conditions, while their relative amount could be associated with the collection technique, conservation methods and also to species behaviour. An environmental effect is also suggested by the spectral similarity of the two *N. commutata* (Nc5 and Nc2), from two different mining regions, that is not observed among the other specimens of this genera out of the mining region.

The LF and HF components characteristic of other insects are present in the spectra. However, neither the spectral area nor the HF/LF intensity ratio of the several ant genera correlate to the local geomagnetic field, in contrast to what was previously observed for *Solenopsis* ants (Wajnberg et al. 2005). The spectral area is proportional

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to the magnetic material amounts, but it is not necessarily associated with magnetic sensitivity as reported for *Atta* ants (Riveros et al. 2014). Instead, the size and arrangement of the particles, which are related to the LF and HF components, and magnetic anisotropy are important parameters for magnetic field sensitivity based on biological magnetic-torque transducers of magnetite (Winklhofer and Kirschvink 2010). The expected increasing HF/LF intensity ratio observed for *Solenopsis* spectra with increasing geomagnetic intensity (Wajnberg et al. 2005) was not observed for any of the studied genera.

As the anisotropic spectral property is a characteristic of workers of *A. colombica* soil nest (Riveros et al. 2014), this spectral anisotropic characteristic, and the presence of the LF component, were then used to group the species studied that could be associated to the raid and nomadic behaviour. The unexpectedly low fraction of anisotropic spectra (about 40%) is probably related to the sample set diversity. A higher percentage (70%) was observed for the more homogeneous sample of *A. colombica* forager ants from a single soil nest, collected once. Even so a distribution in the spectral characteristics was observed. Strikingly, the combination of FMR and a behavioural experiment of this *A. colombica* colony suggested the correlation of the spectra anisotropy with sensitivity to the magnetic compass (Riveros et al. 2014). Unfortunately, the number of specimens at hand was insufficient for calculation of the correlation between spectral anisotropy and the geomagnetic field parameters.

The cluster analysis showed a similarity among the analysed migratory and army ant species that could be related to their common long action radius, making long trials inside the forest floor (Wilson 1971; Mill 1982) during foraging and nest emigration. This resulted in an unexpectedly higher similarity between the *Eciton* army ant and the *Neoponera* migratory ant than between the two army ants, *Eciton* and *Labidus*, although the former are of different phylogenetic origin (Schmidt and Shattuck 2014). Despite the common nomadic behaviour of army ants, they differ in the raiding behaviour. *E. hamatum* and *E.burchelli* raids are diurnal (Franks and Fletcher 1983; Teles da Silva 1982) while *L. praedator* column raiding appeared be larger and more densely populated at night on Barro Colorado Island. Instead, *L. coecus*, raids can be both diurnal and nocturnal (Powell and Baker 2008). Moreover, the epigaeic (aboveground) or hypogaeic (underground) raid character is distinct for these genera. *L. coecus* raids are hypogaeic (Wetterer and Snelling 2015) while *L. praedator* raids are either hypo or epigaeic. In contrast, *N. commutata* as well as *Eciton* species present mostly epigaeic (Mill 1984). Magnetic orientation can be essential for subterranean or nocturnal raids, as in the *Labidus* species studied so that the magnetic proprieties of their particles would be unlike those of the analysed *Eciton* and *Neoponera* species.

It is interesting to note that magnetic data resulted in high dissimilarity between *Mayaponera* and *Neoponera* (Fig. 4). These two genera were initially from the same *Pachycondyla* genus and were recently reclassified as *M. constricta* and *N. commutata. N. commutata* is migratory and makes long group-foraging journeys looking for *Syntermes spp.* termite nests during the day, whereas *M. constricta* conduct nocturnal foraging (Schmidt and Shattuck 2014) and for them long-distance displacements are not described in the literature, as far as we know. The observed differences in the magnetic particle properties could be related to the above behavioural and environmental characteristics. The magnetic particles properties of the studied ants appear not to be related to the phylogenetic similarities once the army ants, *Labidus and Eciton* genera, were distinguished one from each other and those of *Eciton* are closer to those of *Neoponera*; this indicates, however, that raiding behaviour might be the differentiation factor.

Conclusion

The number of species studied in this work contributes to the data on properties of the magnetic particles in ants and suggests that they are related to their adaptive capability. It encourages further studies on magnetic sensitivity in ants and other social insects.

This paper introduces a possible method of classifying biomineralized magnetic material of social insects, which at this stage should be considered as a starting point. The reported findings may stimulate new hypotheses for magnetoreception considering the different dislocation distances in behaviours such as homing and raiding or emigration. Care should be taken with factors, such as environmental condition, conservation and nest function. These studies will demand the following challenging controlled conditions: collection of a large number of workers, preferentially foragers, of the same nest at the same period; analysis of different close nests and a number of species from one genus; more than one genus, if feasible; and the time interval between collecting and measurement must be as short as possible, respecting the conservation method.

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