



Antennae: the strongest magnetic part of the migratory ant

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Abstract

Pachycondyla marginata (*P.m.*), a migratory and termitophagous ant, hunting only the termite species *Neocapritermes opacus*, migrates significantly oriented 13° with respect to the magnetic North-South axis. Results of hysteresis curves at room temperature of four *Pachycondyla marginata* heads, thorax, pairs of antennae and abdomens, oriented parallel to the magnetic field, indicate that the antennae give the strongest saturation magnetization, suggesting this sensory organ as being also a magnetic sensory organ. The total saturation magnetization in a whole *P.m.* is composed by $42 \pm 3\%$, $24 \pm 3\%$, $19 \pm 3\%$ and $15 \pm 3\%$ of antennae, head, thorax and abdomen contributions, respectively. The abdomen hysteresis curve presents a wasp-waisted loop with H_{cr}/H_c of 4.75, characteristic of mixed magnetic systems.

Introduction

Pachycondyla marginata (*P.m.*) ants, common in the southeast region of Brazil, present two interesting characteristics: they are termitophagous ants, exclusively preying on the live termite species *Neocapritermes opacus* (*N.o.*), 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, it was shown that migrations during the dry/cold season are significantly oriented 13° with respect to the magnetic North-South axis, suggesting influence of the geomagnetic field on the choice of migration route (Acosta-Avalos *et al.* 2001). The size distributions of chemically purified magnetic materials of their head, thorax and abdomen were obtained by electron microscopy (Acosta-Avalos *et al.* 1999). EPR analysis (Wajnberg *et al.* 2000) of their macerated abdomen supports a model of isolated magnetite nanoparticles of ~ 13 nm in diameter and indicates the presence of clusters of three of these nanoparticles on the average. Induced remanent magnetization (IRM) loss (Wajnberg *et al.* 2001) yields an upper limit of 1.8×10^9 superparamagnetic (SPM) particles while 1.4×10^8 single domain (SD) particles or aggregates were obtained from room temperature

(RT) IRM values in these macerated abdomens. In this paper RT hysteresis curves were performed to magnetically characterize the magnetic material in each intact part of (*P.m.*) ants, oriented parallel to the magnetic field.

Materials and methods

Pachycondyla marginata (Roger) ants were collected in the region of Campinas, São Paulo, Brazil, and preserved in ethanol 70% (v/v), after being washed in the same solution. The ants were separated into four parts: pair of antennae, head, thorax and abdomen. Each of these parts was mounted on a capton tape, which maintains each unit very close to each other. The samples were oriented in a way that the long body axis would be parallel to the magnetic field. Room temperature hysteresis measurements of these four samples were obtained with a Quantum Design MPMS-XL SQUID magnetometer. The coercivity of remanence H_{cr} value was obtained after a 10 kOe saturating field followed by minor hysteresis loops with decreasing fields from zero to -500 Oe. Curves were fitted with the program Origin.

Results

There is no report on magnetic properties of iron containing proteins, ferritin and hemosiderin in insects, particularly in social insects. However, mammalian proteins were widely studied. Human liver ferritin magnetization is negligible at temperatures higher than 60 K (Dubiel *et al.* 1999). The antiferromagnetic (AFM) ordering temperature of horse spleen ferritin and similarly of hemosiderin was reported to be approximately 240 K (Bell *et al.* 1984, Bauminger and Nowik 1989). Very low AFM ordering temperatures (20 K and approximately 3 K) were determined by Mössbauer spectroscopy for two bacterioferritins (Watt *et al.* 1986, St Pierre *et al.* 1986). At room temperature it is then assumed that iron containing proteins contribute only with a paramagnetic phase. This contribution is not observed in the following results or is dominated by a diamagnetic one as shown below.

Figures 1 and 2 present RT hysteresis curves obtained for oriented samples with four *Pachycondyla marginata* abdomens, thoraxes, pairs of antennae and heads without antennae. Hysteresis of abdomen and thorax presents a diamagnetic contribution, observed as a negative straight line slope appearing at very strong fields, due to the significant non ferro(i)magnetic biological material contribution. The diamagnetic susceptibility contribution to magnetization was obtained by a linear fit of the curve for magnetic fields beyond ferromagnetic saturation and was subtracted. A remarkable point is the strongest magnetization saturation of the pairs of antennae, as compared to the other samples. The total magnetization saturation in a *P.m.* is composed of contributions of $42 \pm 3\%$, $24 \pm 3\%$, $19 \pm 3\%$ and $15 \pm 3\%$ from pair of antennae, head, thorax and abdomen, respectively. These percentages change to $68 \pm 30\%$, $22 \pm 8\%$, $5 \pm 2\%$ and $3 \pm 3\%$ if the magnetization saturation per mass of body part is considered. A wasp-waisted hysteresis is observed only in the abdomen part (easily observed in Figure 2), indicating mixed magnetic systems (Roberts *et al.* 1995), containing, at least, two different magnetic components with strongly contrasting coercivities or mixtures of grain sizes of a single magnetic mineral or a combination of these two situations. A Boltzman sigmoid is proposed to phenomenologically describe the complex ferro(i)magnetic component as it is still challenging to mathematically describe hysteresis curves (Bertotti 1998). Abdomen hysteresis branches were treated us-

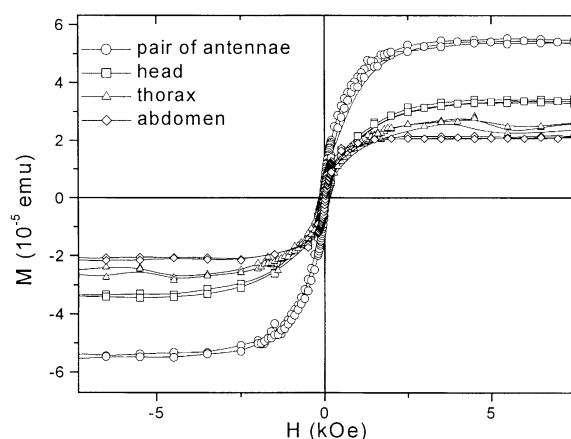


Fig. 1. Hysteresis curves of four *Pachycondyla marginata* heads, abdomens, thorax and pairs of antennae samples oriented parallel to the magnetic field at room temperature.

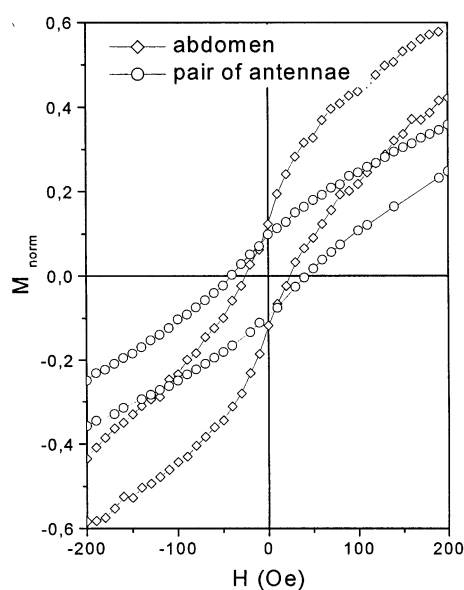


Fig. 2. Details of normalized hysteresis curves abdomen and pair of antennae.

ing two sigmoidal components (labeled as 2 sig in Table 1) or one sigmoid function added to a Langevin one (sig+Lan), with the fraction of each magnetic grain size population obtained by the weighted fitting curve. For the first one, the highest coercive force (H_c) curve contributes 81% to the saturation magnetization with H_c of 64 Oe; this fraction increases to 91% with H_c of 51 Oe when the superparamagnetic contribution is assumed to be described by a Langevin curve. Both curves fit experiment equally well within the experimental error (not shown).

Table 1. Room temperature hysteresis properties of four *P.m.* parts and the two fitted abdomen components (m is the average mass of one body part).

	Antennae	Head	Thorax	(exp)	Abdomen (2 sig)*		(sig+Lan)*	
M_s (10^{-5} emu)	5.4 ± 0.1	3.2 ± 0.2	2.5 ± 0.3	2.0 ± 0.3	1.7	0.4	1.9	0.2
H_c (Oe)	42 ± 1	52 ± 5	61 ± 3	24 ± 1	64	8	51	–
M_r (10^{-6} emu)	5.4 ± 0.3	3.0 ± 0.2	3.0 ± 0.5	2.6 ± 0.3	2.2	0.6	2.5	–
M_r/M_s	$0.10 \pm .01$	$0.09 \pm .02$	$0.13 \pm .03$	$0.13 \pm .03$	0.13	0.15	0.13	–
$m(10^{-3}$ g)	0.5 ± 0.1	0.9 ± 0.1	1.8 ± 0.2	2.5 ± 0.2				
M_s/m^{**} (10^{-2} emu/g)	2.8 ± 0.6	0.9 ± 0.2	0.2 ± 0.05	0.2 ± 0.1				

*See text for details.

**Magnetization per mass of body part: It is not a material property because the magnetic material mass is not known.

Table 1 presents the RT hysteresis properties: saturation magnetization M_s , remanent magnetization M_r and coercive force H_c of thorax, pair of antennae, heads without antennae and abdomen, including those obtained from the abdomen data fit. The H_c values vary from 24 Oe for abdomen to 61 Oe for thorax. The low field region of the antennae and abdomen normalized hysteresis curves are shown in Figure 2. Although the abdomen presents the lowest M_r value, which is about half that of the pair of antennae, the normalized values M_r/M_s are almost the same within experimental error. The four coercivity values are in the range 1200–1500 Oe, indicating that high coercivity minerals like hematite or greigite are not present.

Discussion

Previous results on electron spectroscopic microscopy (Acosta-Avalos *et al.*, 1999) presented the existence of purified magnetic nanoparticles of iron oxides extracted from *P.m.* ant, with different size distributions inside head with antennae, abdomen, and, in relatively low quantity, in thorax. These data clearly showed bimodal distributions in thorax and head with antennae, and the existence of a two size population was suggested, fitting SD and SPM regions according to the magnetic stability diagram for rectangular particles introduced by Butler and Banerjee (1975). The thorax hysteresis curve does not indicate magnetic mixtures, in contrast to the electron spectroscopic microscopy data above, which suggested a result of chemical modification of the original material. Nevertheless, the different head and pair of antennae hysteresis curves obtained independently support the corresponding electron spectroscopic microscopy data. The M_r/M_s and H_c values are close to those

obtained for Pseudo Single Domain (PSD) or Multi Domain (MD) magnetite which exhibit M_r/M_s ratios in the range from 0.162 to 0.008 and H_c from 87 to 12 Oe (Table 2 in Roberts *et al.* 1995).

Although the selected area diffraction (SAD) from chemically isolated abdomen nanoparticles points to magnetite and possibly maghemite as components, a two size distribution was not considered (Acosta-Avalos *et al.* 1999).

On the other hand, EPR results of macerated abdomens were associated with magnetite particles with two different sizes. In this paper the use of intact body parts yields information close to in vivo conditions. Care should be taken in sample preparation that could be responsible for the apparent contradictory results. Hysteresis deconvolution, as presented in Table 1, using a very low H_c component or a Langevin function strengthens the SPM contribution while the other H_c and M_r/M_s component values follow the magnetite PSD or MD region. Moreover, the experimental low $H_c = 24$ Oe and M_r/M_s values and the H_{cr}/H_c ratio of 4.75 ($H_{cr} = 114 \pm 5$ Oe) indicate that the remanence is dominated by MD behavior (table 3 in Roberts *et al.* 1995) with the H_c reduced to 24 Oe by the SPM fraction in the mixture, in good agreement with the 90% (81%) of the MD composite loop in the fit.

Magnetic particles with different size distributions present in the ant body parts studied would have different magnetic properties, which may be related to different biological processes, a complex task to be carried out. There is no model proposed for ants yet, but the double nature of iron compounds, SPM combined with amorphous iron phosphate/calcium complexes in the upper beak of homing pigeon, recently proposed as appropriate detectors of the geomagnetic changes is

an interesting model to be considered (Fleissner *et al.* 2003; Davila *et al.* 2003).

The magnetic material distribution in the migratory ant body is distinct from that found in other social insects. Magnetic material in *Apis mellifera* was studied in whole bees (Gould *et al.* 1978) and most of the material was found in the front third of the abdomen (Kirschvink & Gould 1981); only paramagnetism was reported in the abdomen (Takagi 1995). Induced remanent magnetization of *Nasutitermes exitiosus* termite, fed on pure cellulose, showed 23% of the magnetic material in the head and the remaining 77% in their body (thorax+abdomen), also without considering the antennae contribution (Maher 1998). The magnetometric analysis of the various ant body parts aims at the localization of magnetic materials that might indicate the possible presence of a magnetoreceptor. Surprisingly, all of the four body parts explored in this study display ferro(i)magnetic material, even if it is considered that thorax and abdomen may contain ingested or metabolized particles (Keim *et al.* 2002; Nichol *et al.* 2002). Particularly interesting is the relative high fraction of ferro(i)magnetic material present in the pair of antennae, where only biomineralized particles can be present. This sensory organ is connected to the deutocerebrum, known for olfactory and tactile functions. These results suggest antennae as a possible magnetic sensory organ in the migratory ant, pointing to the necessity of innumerable additional behavioral, physical and physiological experiments.

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References

- Acosta-Avalos D, Esquivel DMS, Wajnberg E, Lins de Barros HGP, Oliveira PS, Leal IR. 2001 Seasonal patterns in the orientation system of the migratory ant *Pachycondyla marginata*. *Naturwissenschaften* **88**, 343–346.
- Acosta-Avalos D, Wajnberg E, Oliveira PS, Leal IR, Farina M, Esquivel DMS. 1999 Isolation of magnetic nanoparticles from *Pachycondyla marginata* ants. *J Exp Biol* **202**, 2687–2692.
- Bauminger ER, Nowik I. 1989 Magnetism in plant and mammalian ferritin. *Hyp Int* **50**, 484–498.
- Bell SH, Weir MP, Dickson DPE, Gibson JF, Sharp GA, Peters TJ. 1984 Mossbauer spectroscopic studies of human haemosiderin and ferritin. *Biochim et Biophys Acta* **787**, 227–236.
- Bertotti G. 1998 Hysteresis in magnetism, San Diego: Academic Press.
- Butler RF, Banerjee SK. 1975 Theoretical single-domain grain size range in magnetite and titanomagnetite. *J Geophys Res* **80**, 252.
- Dubiel SM, Zablotna-Ryplen B, Mackey JB, Williams JM. 1999 Magnetic properties of human liver and brain ferritin. *Eur Biophys J* **28**, 263–267.
- Gould JL, Kirschvink JL, Deffeyes KS. 1978 Bees have magnetic remanence. *Science* **201**, 1026–1028.
- Kadir FHA, Read NMK, Dickson DPE, Greenwood C, Thompson A, Moore GR. 1991 Mossbauer spectroscopic studies of iron in *Pseudomonas aeruginosa*. *J Inorg Biochem* **43** (4), 753–758.
- Keim CN, Cruz-Landim C, Carneiro FG, Carneiro FG, Farina M. 2002 Ferritin in iron containing granules from the fat body of the honeybees *Apis mellifera* and *Scaptotrigona postica*. *Micron* **33** (1), 53–59.
- Kirschvink J, Gould JL. 1981 Biogenic magnetite as a bases for magnetic field detection in animals. *Biosystems* **13**, 181.
- Leal IR, Oliveira PS. 1995 Behavioral ecology of the neotropical termite-hunting ant *Pachycondyla* (= *Termitopone*) *marginata*: colony founding, group-raiding and migratory patterns. *Behav Ecol Sociobiol* **37**, 373–383.
- Maher BA. 1998 Magnetite biomineralization in termites *Proc R Soc London B* **265**, 733–737.
- Roberts AP, Cui Y, Verosub KL. 1995 Wasp-waisted hysteresis loops: Mineral magnetic characteristics and discrimination of components in mixed magnetic systems. *J Geophys Res* **100** (9), 17909–17923.
- Takagi S. 1995 Paramagnetism of honeybees. *J Phys Soc Jap* **64**, 4378–4781.
- St. Pierre TG, Bell SH, Dickson DPE, Mann S, Webb J, Moore GR, Williams RJP. 1986 Mossbauer spectroscopy studies of the cores of human, limpet and bacterial ferritins. *Biochim Biophys Acta* **870**, 127–134.
- Wajnberg E, Acosta-Avalos D, El-Jaick LJ *et al.* 2000 EPR study of migratory ant *Pachycondyla marginata* abdomens. *Biophys J* **78** (2) 1018–1022.
- Wajnberg E, Cernicchiaro G, Acosta-Avalos D, El-Jaick LJ, Esquivel DMS. 2001 Induced remanent magnetization of social insects. *J Mag Mat Mag* **226–230**, 2040–2041.
- Watt GD, Frankel RB, Papaefthymiou GC, Spartalian K, Stiefel EI. 1986 Redox properties and Mossbauer spectroscopy of *Azotobacter-vinelandii* bacterioferritin. *Biochemistry* **25** (15), 4330–4336.