ZFC/FC of oriented magnetic material in the *Solenopsis interrupta* head with antennae: Characterization by FMR and SQUID

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Abstract Ferromagnetic resonance and SQUID magnetometry have been used to study magnetic material in the head with antennae, thorax, and abdomen of *Solenopsis interrupta* ants. The temperature dependence of the head with antennae using both techniques was measured. Room-temperature spectra and saturation magnetization were used to compare the magnetic material amount in the ant body parts. Both techniques show that the highest magnetic material fraction is in the head with antennae. The ordering temperature is observed at 100 ± 20 K for the ferromagnetic resonance spectra HF component. The estimated magnetic anisotropy constant K and g -values at room temperature are in good agreement with magnetite, supporting this material as the main magnetic particle constituent in the *Solenopsis interrupta* head with antenna. Particle diameters of 26 ± 2 nm and smaller than 14 nm were estimated. This work suggests that the head with antenna of the *Solenopsis interrupta* ant contains organized magnetic material and points to it as a good candidate as a magnetic sensor.

Keywords Fire ants · FMR · SQUID · Magnetic nanoparticles · Temperature transitions

1 Introduction

Behavioral experiments have shown that several animal species use the geomagnetic field as an orientation cue [1–4]. Since then, a few theoretical models have been developed in order to understand how organisms orient using the geomagnetic field [3, 5–8] and there has been

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an increase in experiments looking for the magnetic sensor [9–16]. Two main hypotheses of magnetic orientation have been proposed. The ferromagnetic hypothesis based on magnetic particles and the light-dependence hypothesis based on chemical reactions with radical pair formation [4, 8].

Based on the ferromagnetic hypothesis, the magnetic material in social insects has been studied [9–16]. The magnetic moment was suggested to develop in the pupae state persisting in the adult of *Apis mellifera* honey bees [17], indicating that the magnetic material is constructed in an orderly way that might well suit it for use as a detector and points to a correlation between the magnetic material amount and function. Magnetite nanoparticles of 30–35 nm diameter in these bee abdomens [18] were assumed to be involved in magnetic field detection due to their stability. Later on, electron-dense material was found in the second abdominal ganglion of these bees, as a single domain and superparamagnetic magnetite [19]. The presence of superparamagnetic magnetite in iron granules formed in honeybees was ascribed to the notion that external magnetic fields may cause expansion or contraction of these particles in an orientation-specific manner, relaying the signal via cytoskeleton [20, 21], initiating a neural response.

Ants have also become an insect of interest, as an orientation system is used in homing after foraging. A magnetic compass response has been shown for *Acromyrmex octospinosus* [22], *Formica rufa* [23], *Formica pratensis* [24], and *Oecophylla smaragdina* foraging [25]. *Atta colombica* ants respond to magnetic reversal in the absence of sunlight cues [26]. Stimulated by the observation of the magnetic field effect on the time for trail formation of *Solenopsis invicta* ants [27], the presence of magnetic material in a *Solenopsis* ant genus was shown by ferromagnetic resonance (FMR) [9].

Magnetic material identification, localization, and physical characterization contribute to understanding the magnetoreception mechanism based on the ferromagnetic hypothesis. Ferromagnetic resonance (FMR) was shown to be a useful technique for localizing and characterizing magnetic material in ants, such as smashed *Solenopsis* sp. ants [9], smashed *Pachycondyla marginata* abdomens [10], *Solenopsis substituta* body parts [13] and *S. interrupta* body parts [15, 16].

Few FMR and SQUID magnetometry studies on magnetic materials in the body parts of social insects have been performed [12–16]. FMR studies of smashed insect body parts yield interesting results on magnetic material amounts, volume and magnetic anisotropy estimates [10, 11, 13]. Nevertheless, the sample preparation does not preserve the natural organization of this material and can expose it to chemical reactions because of body disruption. Studies on oriented intact body parts at 300K have shown that the major magnetic contribution comes from the antennae in the ant *P. marginata* [12], the stingless bee *Schwarziana quadripunctata* [14] and the ant *S. interrupta* [15, 16]. Further, the magnetic material found on the *P. marginata* ant Johnston's organ and other joints (localized in the antennal pedicel) was suggested as a good candidate as a magnetic sensor by Oliveira et al. [28].

Abraçado et al. [16] showed that the FMR line shapes of *S. interrupta* ant body parts are reproducible and they used 25 oriented samples to estimate statistically the relative magnetic material amount on each body part ($31 \pm 12\%$ in the antennae, $27 \pm 13\%$ in the head, $21 \pm 12\%$ in the thorax and $20 \pm 10\%$ in the abdomen). The angular dependence of the resonant field was observed only in the heads and antennae of *S. interrupta*, which suggests the presence of an oriented and biogenic magnetic material in these body parts [15]. While the idea that the magnetic material can be ingested from the soil by ants cannot be discarded, if true this material would only be found in the thorax and abdomen. The reproducibility of FMR line shapes, relative magnetic material amount and the presence of

oriented magnetic material in the head and antennae body parts makes the *S. interrupta* ant an interesting subject for the investigation of a magnetic sensor.

In this paper, FMR and SQUID at room temperature (RT) were used to quantify the relative amount of the magnetic material in body parts of the *S. interrupta* ant, which was compared with previous data in the literature [15, 16] in order to prove that our sample body parts have a relative amount of magnetic material compatible to the expected limit for this ant. Further, the temperature dependence of oriented heads with antennae was performed to verify the spatial organization of the particles and estimate their physical properties. Information on the magnetic material orientation can be useful in distinguishing biomineralized from ingested material and in developing magnetoreception models.

2 Materials and methods

S. interrupta ants were collected in Citrolândia, Rio de Janeiro, Brazil. The nest was kept alive in the laboratory by spraying distilled water and feeding the ants with *Nauphoeta cinerea* cockroaches and a Bhatkar diet [29]. The nest was connected to the feeder by a wood bridge, where foraging activity was observed. This system allows the identification of forager ants that carry food. Only ants coming back to the nest carrying food were collected. The ants were kept in glutaraldehyde 2.5% for 24 h, washed and preserved in a 0.1 M cacodylate buffer at pH 7.4, glutaraldehyde 2.5%. They were separated into three parts: head with antennae (HEAD), abdomen with petiole (ABD), and thorax with feet (THO). Just before measurement, the body parts were dried at 50°C for 1 h. Each sample was composed of 20 ant body parts. This number is based on previous results in *S. interrupta* ants [15, 16], which show that a combination of 20 body parts can represent the colony workers. Two different samples were used for FMR and SQUID measurements.

FMR measurements were performed with an X-band EPR spectrometer (Bruker ESP300E) operating at a microwave power of 4 mW with a 100 kHz modulation frequency and a ~ 2 Oe modulation field in amplitude. The temperature dependence of the HEAD sample was performed from 4 K to RT. The HEAD sample was oriented with the applied magnetic field perpendicular (PER) or parallel (PAR) to the long body axis (Fig. 1a and b, respectively) and cooled under a zero (ZFC) and a 3000 Oe (FC) magnetic field.

Microcal Origin 6.0 software was used to fit the spectra to a sum of Gaussian (low-field component, LF) and Lorentzian (overlapped high-field components, HF and HF') lines. The fitting parameters are the resonance line width (ΔH_{pp}), the resonance field (H_R) and the absorption area (A) of the spectral components (Fig. 1c). The A, H_R and ΔH_{pp} errors were estimated by averaging fitting results with a correlation coefficient $R \geq 0.89$. The absorption spectrum areas were calculated as the sum of the A component fitting values and as the second integral of the derivative spectra, S, using a software developed on the graphic LabVIEW® platform, starting at high field values where the baseline is clearly defined.

HEAD hysteresis curves, from 5 to 300 K, were obtained with a SQUID magnetometer (Quantum Design MPMS-X) with THOR and ABD only at 300 K, in PAR orientation. These insect tissues present paramagnetic and/or diamagnetic contributions, observed as a positive or negative straight line at high field ranges. The resultant paramagnetic and diamagnetic susceptibilities were obtained by the linear fit of the curve at magnetic fields beyond ferromagnetic saturation and the contribution was then subtracted to obtain the hysteresis loop.

Fig. 1 **a** Ant oriented with the applied magnetic field perpendicular (PER) and **b** parallel (PAR) to the long body axis. **c** Fitting parameters of the spectrum components. **d** Ferromagnetic resonance (FMR) spectra of body parts, oriented perpendicular to the magnetic field at RT

3 Results

3.1 FMR

Figure 1d shows the RT FMR spectra of the ant body parts in PER orientation. The absorption curve areas, S and A , given in Table 1, are proportional to the magnetic material amount [13–16]. The magnetic material amount estimated before for *S. interrupta* ant body parts was obtained in PER orientation using the absorption curve area S [15, 16]. The relative magnetic material fractions of each body part were obtained and are also given in Table 1. The values obtained from A differ from S because they do not account for the whole LF component as parameter A does, as it is related to the fitting curve. Both results show a larger amount of magnetic material in the HEAD.

The temperature dependences of the HEAD spectra in PER orientation under FC and ZFC conditions are shown in Fig. 2a and b, respectively. Similar spectra were obtained in PAR orientation (not shown). Fitting examples are shown for the FC at RT and ZFC spectra at 4 K. A narrow line ($\Delta H \sim 14$ Oe) at $g = 2$, related to free radicals resulting from biological processes, is easily observed in spectra under the FC condition. A line (HF')

Table 1 Hysteresis and FMR spectrum parameters of *Solenopsis interrupta* ant body parts at room temperature

Sample\Parameter	HEAD	ABD	THOR
Magnetic material amount from S ($\times 10^9$ a.u.)	5.2 ± 0.5	1.5 ± 0.2	2.2 ± 0.3
Magnetic material fractions from S	$58 \pm 9\%$	$17 \pm 3\%$	$25 \pm 4\%$
Magnetic material amount from the sum of fitting parameters A ($\times 10^{10}$ a.u.)	6.6 ± 0.3	0.45 ± 0.02	1.1 ± 0.2
Magnetic material fractions from A	$81 \pm 6\%$	$6 \pm 1\%$	$13 \pm 3\%$
J_S ($\times 10^{-5}$ emu)	1.6 ± 0.4	0.52 ± 0.16	0.31 ± 0.04
J_R ($\times 10^{-6}$ emu)	1.3 ± 0.5	0.53 ± 0.04	0.19 ± 0.04
H_C (Oe)	13 ± 5	83 ± 8	83 ± 2
$\frac{J_R}{J_S}$	0.08 ± 0.04	0.1 ± 0.03	0.06 ± 0.01
Magnetic material fractions from J_S	$66 \pm 23\%$	$21 \pm 8\%$	$13 \pm 4\%$

with about 100 Oe width and $g = 2.066$, which presents typical paramagnetic behavior, is resolved below 70 K. Two more components with line widths larger than 500 Oe are observed. One around $g = 2.0$ (HF), easily observed at high temperature, shifts to $g = 3.2$ at 4.5 K (~ 500 Oe line width). The second one (LF) is observed at $g = 4.3$ at RT and shifts to $g = 3.8$ at 4 K with a ~ 2000 Oe line width. The LF line is observed by the non-zero baseline at low fields.

The H_R temperature dependences of HEAD HF and LF components are shown in Fig. 3. The H_R of the HF component curves saturate at temperatures higher than 130 K with values in the range of 3220 ± 50 Oe and 3010 ± 50 Oe for ZFC in PAR and PER orientation,

Fig. 2 Temperature dependence of the head with antennae spectra in PER orientation **a** cooled under a zero (ZFC) and **b** cooled under a 3000 Oe (FC) magnetic field. Fitting examples are shown for the FC spectrum at RT and ZFC spectrum at 4 K

Fig. 3 Temperature dependence of the resonant field H_R , **a** PER orientation in ZFC ($\blacktriangle$ -HF, ∇ -LF) and FC (Δ -HF, ∇ -LF) conditions, **b** PAR orientation in ZFC ($\blacksquare$ -HF, $\bullet$ -LF) and FC ($\square$ -HF, $\circ$ -LF) conditions, **c** PER ($\blacktriangle$ -HF, ∇ -LF) and PAR ($\blacksquare$ -HF, $\bullet$ -LF) orientations in ZFC condition and **d** PER (Δ -HF, ∇ -LF) and PAR ($\square$ -HF, $\circ$ -LF) orientations in FC condition. Solid lines are guides to the eyes. Error bars are shown for data with error larger than symbols. Errors were estimated by averaging fitting results with a correlation coefficient $R \geq 0.89$

respectively, and 3090 ± 50 Oe for FC in both orientations. The related g limit values at the high temperature range are 2.08 ± 0.03 and 2.25 ± 0.01 for ZFC in PAR and PER orientation, respectively, and 2.17 ± 0.03 for FC in both orientations.

The H_R temperature dependence of the HF and LF components is similarly independent of the sample orientation and of the cooling condition. The PER curves present a clear shift from the ZFC to FC conditions (Fig. 3a), as well as the HF curve in PAR orientation (Fig. 3b), while the LF curves are indistinguishable for $T > 20$ K within the experimental error. The orientation effect on the LF and HF H_R curves is observed in the ZFC (Fig. 3c) but in the FC conditions the curves are almost coincident (Fig. 3d).

Isolated spherical nanoparticles were associated with the HF component without H_D contribution, while the LF was associated with large particles or clusters. Based on the parallel behavior of the LF and HF H_R curves above 180 K, the demagnetization field (H_D) and the anisotropy field (H_A) are estimated considering Eq. (1), as previously considered for other insects [10, 11, 13]:

$$\hbar\omega_0 = g\beta H_{eff} = g\beta (H_R + H_D + H_A). \quad (1)$$

The H_D is then calculated by the difference between the LF and HF values of H_R in the temperature range where the curves are parallel. H_D values are 1300 ± 50 Oe and 1450 ± 40 Oe in PAR orientation, for FC and ZFC conditions, respectively. The mean value of $H_D = 1400 \pm 100$ Oe is taken considering the four conditions. The high temperature g values estimated before are in good agreement with bacterial magnetite, $g = 2.01$ – 2.25 [30], bulk magnetite, $g = 2.12$ [31–33] and magnetite nanoparticles, $g = 2.25$ [34]. Magnetite ($M_S = 470$ G) is then considered as the constituent material, so that the H_D value matches $q = 2$. Assuming a prolate ellipsoid with three principal geometrical axes (a , b and c) satisfying $c \gg a = b$ and $q = c/b$, the dimensional ratio suggests a nanoparticle dimer or a particle of equivalent shape [10, 13, 35]. Similar results are obtained if maghemite ($M_S = 420$ G) is considered.

The H_A was calculated using each limiting g -value and the H_D average value in (1). The results are shown in Fig. 4, based on Belov [36] and T_{or} and T_t are indicated. The magneto-orientation (T_{or}) is characterized by null H_A and the transition temperature T_t is related to a jump in the anisotropy constant. The T_{or} of the HF components (Fig. 4) is observed at 100 ± 20 K in all conditions. The T_{or} of the LF component is observed at 70 ± 5 K in PER orientation under ZFC and at 40 ± 10 K in the other conditions. T_t is observed only for the LF component at 20 ± 10 K in the FC condition and PAR orientation and at 40 ± 10 K for the three other conditions.

The temperature dependences of the HF and LF line widths are shown in Fig. 5. The curves are non-monotonic with minima and maxima. The FC clearly affects the line width values in PER orientation.

3.2 SQUID magnetometry

The HEAD and THOR hysteresis curves present a resultant diamagnetic contribution, while the ABD hysteresis is dominated by a paramagnetic contribution. Figures 6a and b show the hysteresis curves of ant body parts at 300 K (after the dia/paramagnetic contribution has been subtracted), from which the coercivity field (H_C), the saturation (J_S), the remanent magnetizations (J_R) and the $\frac{J_R}{J_S}$ ratios are obtained (Table 1). The relative magnetic material fractions of each body part obtained from J_S are $66 \pm 23\%$, $21 \pm 8\%$ and $13 \pm 4\%$ for HEAD, ABD and THO, respectively, considering the total J_S as the sum of the J_S body parts,

Fig. 4 H_A temperature dependence obtained from H_R (see text), **a** PER orientation in ZFC (■-HF, ●-LF) and FC (□-HF, ○-LF) conditions and **b** PAR orientation in ZFC (■-HF, ●-LF) and FC (□-HF, ○-LF) conditions. *Solid lines* are guides to the eyes

Fig. 5 ΔH_{pp} temperature dependence, **a** PER orientation in ZFC (■-HF, ●-LF) and FC (□-HF, ○-LF) conditions and **b** PAR orientation in ZFC (■-HF, ●-LF) and FC (□-HF, ○-LF) conditions. *Solid lines* are guides to the eyes. Error bars are shown for data with error larger than symbols. Errors were estimated by averaging fitting results with a correlation coefficient $R \geq 0.89$

Fig. 6 Hysteresis curve of *Solenopsis interrupta* ant body parts oriented parallel to the magnetic field at 300 K (a). Insertion of the low-field region of ant body part hysteresis curves (b). Solid lines are guides to the eyes

2.4×10^{-5} emu. They reveal a higher magnetic material amount in HEAD, in agreement with FMR results (Table 1) and this body part presents a lower H_C than the others.

The H_C and $\frac{J_R}{J_S}$ temperature dependences of the HEAD present a transition at about 180 ± 20 K, indicated as T^* in Fig. 7a and b, respectively. The J_R temperature dependence is shown in Fig. 7c and its derivative, which is proportional to the blocking temperature (T_B) distribution, in Fig. 7d. It presents, at least, a peak at $T_B < 100$ K that is related to a mean particle volume (diameter) of $V < 1.5 \times 10^{-17}$ cm³ ($D < 14$ nm).

Considering a system of nanoparticles with uniaxial anisotropy, the H_C temperature dependence is given by [37]:

$$H_C = H_{C,0} \left[1 - \left(\frac{25k_B T}{KV} \right)^k \right] \quad (2)$$

where $H_{C,0} = \frac{2K}{M_S}$ and k is 0.5 for oriented particles or 0.77 for a random particle system [37]. The factor $\frac{25k_B}{KV}$ is the blocking temperature (T_B), where K is the anisotropy constant. The fitting of the H_C curve, with (2), presents a slightly higher correlation factor ($R = 0.93$) with $k = 0.5$, $T_B = 55 \pm 3$ K and $H_{C,0} = 102 \pm 6$ Oe (solid line in Fig. 7a) than with $k = 0.77$. Taking $M_S = 470$ G, the K value is obtained from $H_{C,0}$ as 2.3×10^4 erg/cm³ and then a volume (diameter) of $(8.3 \pm 0.3) \times 10^{-18}$ cm³ ($D = 26 \pm 2$ nm) is estimated from T_B .

The J_R decrease with increasing temperature is related to the superparamagnetic (SPM) particles and the non-null H_C at RT indicate the additional presence of stable magnetic particles. The SPM particle fraction in HEAD was estimated as 55% using Eq. (3) [38, 39],

$$f_{SP} = \frac{J_R(4.2K) - J_R(300K)}{J_R(4.2K)} \quad (3)$$

Fig. 7 Temperature dependence of **a** coercivity field (H_C), where the *dotted line* is smoothed without the point at 5 K and the *solid line* is the fit with equation (3) for $k = 0.5$, with $T_B = 55 \pm 3$ K and $H_{C,0} = 102 \pm 5$ Oe; **b** correlation J_R/J_S ; **c** J_R and **d** J_R derivative. *Solid lines* in **b** through **d** are added as a guide to the eye. Error bars represent interpolation interval of two consecutive measurements

This fraction can be sub-estimated as J_R at $T = 5$ K and is used instead of $T = 4.2$ K in this equation. In the case of a two size magnetite nanoparticles system, the H_C value depends on the fraction of the low coercivity material as derived by Bean [40]. Taking

the SP+SD branch and $K = 2.3 \times 10^4 \text{ erg/cm}^3$, a $70 \pm 5\%$ SPM fraction is estimated in HEAD. In ABD and THOR, the SPM fraction can be estimated as between 55–91% and 55–75%, respectively.

4 Discussion

A large number of species in the *Myrmicinae* (including *Solenopsis*), *Dolichoderinae*, and *Formicinae* families build trails, deploying workers rapidly into foraging areas. Only the forager ants go out from the nest to explore new places looking for food and then build trails for the other workers [29]. This process demands different orientation mechanisms. One of these mechanisms is magnetic orientation, which involves a magnetic sensor. Two models were developed based on the ferromagnetic hypothesis involving single domains [3, 41] or superparamagnetic magnetite particles [7, 41–43] mediating magnetic information.

A previous work with *S. interrupta* ant body parts showed angular dependence in H_R of head and antennae FMR spectra [15], indicating the presence of organized magnetic material in these body parts. This result strongly suggests specific characteristics for biogenic magnetite. The abdomen and thorax may contain ingested material that is not oriented and would not be appropriate as a magnetic sensor, or it does not allow the magnetic sensor orientation to be revealed [15]. Under a reliable function of the biomineralized particles in the head and antennae, added to the consideration of antennae sensory functions, it is a good candidate as a magnetic sensor organ. Moreover, independent of technique, the highest magnetic material fraction was found in the *S. interrupta* HEAD. This result is supported by statistical work that showed that 25 oriented samples of each body part of *S. interrupta* (20 units each) present reproducible FMR line shapes. The relative magnetic material amount statistically calculated was $31 \pm 12\%$ in the antennae, $27 \pm 13\%$ in the head, $21 \pm 12\%$ in the thorax and $20 \pm 10\%$ in the abdomen [16]. The sum of *S. interrupta* antennae and head magnetic material amount gives a total of $56 \pm 5\%$ [15] and $58 \pm 25\%$ [16]. The largest magnetic material amount in the head with antennae is in good agreement with the result in the smashed body parts of *Solenopsis substituta* ants [13].

The ferromagnetic broad lines associated with magnetite or maghemite isolated nanoparticles and with large nanoparticles or aggregates were observed in insect spectra [9–11, 13–16]. The presence of two components in the FMR spectra points to at least two different particle sizes in *S. interrupta* ant body parts. One of them is associated with superparamagnetic particles, with an estimated 55–70% range in HEAD.

The idea that superparamagnetic particles could be involved in magnetoreception was first suggested by Kirschvik and Gould [44]. At least two types of information can potentially be derived from the Earth's magnetic field. A sense of direction, in animals that have a magnetic compass, relates to single domain particles, while a system called a map sense, which provides the ability to determine position relative to a destination, relates to superparamagnetic particles [2]. Several hypotheses of how magnetite-based receptors may convey magnetic input have been suggested [42–46]. This behavior requires additional magnetic information, such as field polarity and inclination angle. It is possible that the pulse field can affect the transduction of information necessary for the animal to accurately access field polarity, inclination, or both. Considering that the magnetic particles involved in the magnetic sense are not free to align with the ambient field, the animals would receive erroneous information from a subset of receptor cells, likely leading to disorientation. Initially, it was assumed that superparamagnetic particles would not be affected by a pulse

since they would be strongly disturbed by thermal agitation. However, it was shown that superparamagnetic particles occur in groups of between ten and 20 clusters per terminal in the pigeon's tissue [43] and therefore magnetically interact [46]. Interactions between the clusters may cause deformations of the surrounding tissue and the magnetic field can be converted into a mechanical stimulus, which could be transduced into a potential receptor. Models using droplets of ferrofluids as an analogue of superparamagnetic clusters demonstrated the effect of a strong and brief pulse on the chain-like arrangement of the ferrofluid droplets [42].

The H_R temperature dependence curves in PER and PAR orientations became coincident considering the experimental error after applying a 3000 Oe field (FC). A similar coincidence can be observed in FC ΔH_{pp} curves above 50 K. This points to a magnetic moment orientation with the applied magnetic field. The H_R curves in ZFC and FC conditions present a difference of around 200 Oe to HF in PAR orientation, 100 Oe in PER orientation and 200 Oe to LF in PER orientation; there is no change to LF in PAR orientation. This suggests two different magnetic systems with a different orientation of the easy-axis of magnetization. It is possible that the system, represented by the HF component, is perpendicular to the long body axis and the system represented by LF is parallel.

The ΔH_{pp} and H_R temperature dependence of the HF component (isolated nanoparticles) show an inverse behavior, above 80 K for FC, for both orientations. This result has already been observed for different synthetic nanoparticles [47–50]. However, the ΔH_{pp} and H_R temperature dependence of the LF component are almost coincident, independent of orientation and cooling condition.

Magnetite was suggested as the magnetic material present in *P. marginata* smashed abdomens [10] and *S. substituta* ant smashed heads with antennae and abdomens [13]. Based on the *S. interrupta* ant HEAD room temperature spectra, and an anisotropy constant of $K = 2.3 \times 10^4$ erg/cm³, the g-values were estimated. They are in good agreement with magnetite [30–34] being as the magnetic particle constituent in this body part.

The properties of magnetite in the low-temperature transition region from 100 to 135 K are still unclear. The transition temperature, T_t , associated with electronic properties in the 100–120 K range, and the ordering temperature, T_{or} , related to magneto-orientation in the 130–135 K range, where the anisotropy is zero, were proposed [36]. A T_{or} is observed for the HEAD HF components at 100 ± 20 K for all conditions, lower than that discussed by Belov [36]. However, T_{or} estimates strongly depend on the g values, which were extrapolated from the experimental data.

A structure with a diameter smaller than 14 nm was observed through the derivative of the remanence curve fitting. The model used to fit the coercivity field temperature dependence data indicated that the particles with a diameter of 26 ± 2 nm are oriented in this ant body part; however, this model does not consider particle size distribution.

Magnetic techniques had been used to characterize paleomagnetic particles based on systematic grain-size studies as that of crushed titanomagnetite [51]. Considering magnetite, in some cases, it is possible to obtain an indication of the particles' magnetic domain region at RT from the Tauxe et al. [52] diagram ($\frac{J_R}{J_S}$ versus H_c). More recently, the complexity of magnetite systems has necessitated the inclusion of more diagrams and analyses considering basic mixtures of magnetic domains and temperature variation [39, 52–54]. However, this data interpolation with the Tauxe diagram suggests the magnetic measurement parameters are strongly correlated with the particle synthesis protocol. Özdemir et al. [53] analyzed magnetic measurements of magnetite crystals with a mean size of 37, 100 and 220 nm. According to the Tauxe diagram, these particles would be in the cubic single domain region.

A similar disagreement can be observed with magnetite of 5, 10, 50, and 150 nm synthesized by Goya et al. [54]. Particles of 5 and 10 nm are observed in the multi-domain region of the Tauxe diagram, 50 nm in the cubic single domain region and 150 nm in the uniaxial single domain region. Magnetite grains with a narrow distribution and a mixture of multi-domain and pseudo-single domain magnetic measurements analyzed by Roberts et al. [39] are not in agreement with the Tauxe diagram either. Multi-domain particles are in the same region in both cases. However, a mixture of the multi-domain and pseudo-single domain is in the multi-domain region in the Tauxe diagram, pseudo-single domain particles are in the cubic single domain region, and the fine pseudo-single domain in the mixture region of uniaxial single domain and superparamagnetic particles. The interpolation of ant body part parameters in the Tauxe diagram indicated particles in the multi-domain region of HEAD, ABD, and THOR.

5 Conclusions

There is no specific model to study biogenic magnetic particles in insects. Usually, the interpretations are based on models from synthesized inorganic materials [10, 11, 13]. However, these analyzes, based on magnetite, do not consider localization, organization, possible interactions, size distribution or possible effects from biogenic synthesis. The results presented here can give some information about magnetic particles in living beings and point to some special characteristics found in the *S. interrupta* ant HEAD observed before in other insects [10, 11, 13, 14, 18, 19], like two components in the FMR spectra representing at least two different particle sizes, a structure associated with superparamagnetic particles and magnetite as the magnetic particle constituent in these insects. Moreover, the differences in the magnetic moment orientation with the applied magnetic field in parallel and perpendicular orientations suggest the magnetic material is not randomly oriented, in agreement with previous *S. interrupta* ant FMR results [15]. It is the first result in the *S. interrupta* ant, obtained by two techniques, which specifies a characteristic that could be related to biological function, probably the magnetic sensor in magnetoreception. The magnetic material orientation inside *S. interrupta* ants is still unclear; however, the possibility of the characteristics discussed before to be correlated with some kind of orientation cannot be discarded.

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