BioMetals (2006) 19:295–300
DOI 10.1007/s10534-005-0520-4

Stingless bee antennae: A magnetic sensory organ?

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Received 14 April 2005; accepted 5 July 2005

Key words: stingless bee, pair of antennae, SQUID, FMR

Abstract

Magnetic material in the body parts of the stingless bee *Schwarziana quadripunctata*, heads, pairs of antennae, thorax and abdomens, were investigated by SQUID magnetometry and Ferromagnetic Resonance (FMR). The saturation, J_s and remanent, J_r, magnetizations and coercive field H_c are determined from the hysteresis curves. From H_c and J_r/J_s the magnetic particle sizes are estimated. The J_s and the FMR spectral absorption areas yield $23 \pm 3\%$, $45 \pm 5\%$, $15 \pm 2\%$ and $19 \pm 4\%$ magnetic material contributions of head, pair of antennae, thorax and abdomen, respectively, similar to those observed in the migratory ant *Pachycondyla marginata*. This result is discussed in light of the hypothesis of antennae as a magnetosensor structure.

Introduction

For the last 30 years, since the evidence of magnetotactic bacteria magnetosomes containing magnetite biomineralized nanoparticles (Blakemore 1975), several works on different fields have been developed in order to understand geomagnetic orientation in organisms. Behavioural experiments were performed involving several species of animals (Wiltschko & Wiltschko 1995; Vácha & Soukopová 2004; Wiltschko *et al.* 2004) and pursuing the comprehension of the mechanism underneath this phenomenon. In particular, extensive studies on insects have been focused on the honeybee *Apis mellifera*. The correlation between honeybee behaviour and the geomagnetic field was firstly proved in 1968 (Lindauer & Martin 1968). Later on, magnetic material was detected in their body using superconducting magnetometers and pointing to a putative mechanism made of minute particles acting as a magnetic sensor (Gould *et al.* 1978). Iron-containing trophocytes were found within the fat body of this adult honeybee (Kuterbach & Walcott 1986), identified as superparamagnetic (SPM) magnetite particles (Hsu & Li 1994), although this result was not reproduced. Electron-dense material found in the hairs of honeybee abdomens or near the cutex was proposed as single domain or SPM magnetite (Schiff 1991) and a hypothesis was developed for associative learning of visual and magnetic stimuli (Schiff & Canal 1993). The presence of iron particles were also observed by optical and electron microscopy in the trophocytes of adult *Scaptotrigona postica*, a stingless honeybee (Cunha *et al.* 1987). More recently, iron-rich granules found in the fat body of queen honeybees *A. mellifera* and *S. postica*, were proposed to be formed by holoferritin molecules with inorganic phosphate and calcium (and magnesium in *S. postica*) with diameters smaller than those previously described in the literature (Keim *et al.* 2002).

A motivation for searching such a sensor would be the confirmation that the species behaviour is sensitive to the geomagnetic field. The first steps are to detect and localize magnetic nanoparticles as candidates for magnetic receptors, determining their magnetic properties. The following step, more complex, is to understand the physiological process that is involved in the magnetoreception mechanism. This seems to be the case of the *Schwarziana quadripunctata* bee for which the magnetic field effect was observed in the frequency of nest exiting (Nascimento *et al.* 2001).

In this report we present room temperature (RT) SQUID magnetic measurements and ferromagnetic resonance technique (FMR) results for magnetic material in the body parts of the *S. quadripunctata* bee, aiming to existence of a magnetoreceptor.

Methods and materials

The meliponini stingless bee *S. quadripunctata*, native of the Atlantic Forest, was found in an underground nest located at Teresópolis, Rio de Janeiro-Brazil, at 1000 m above the sea level and geomagnetic field intensity 0.238 Oe, inclination −32° and declination −20°30′. Adult foragers were collected in the summer between 8–13 h, a period of maximum foraging activity within the optimal flying temperature range of 21–26 °C (Imperatriz-Fonseca & Darakjian 1994). Bees were collected still alive, put in a refrigerator and after a week transferred to cacodylate buffer 0.1 M pH 7.4. Ten individuals were used without thoraxical members. Two groups of four bees each were separated in four parts: head, pair of antennae, thorax and abdomen, for SQUID and FMR experiments. To minimize contamination, stainless-steel instruments were used. Two whole bees were kept for control. The SQUID sample holder does not fit more than two individuals.

Just before measurements, samples were dried at 50 °C for 1 h. Four units of each body part were oriented one unit close to each other fixed on a kapton tape and on a Teflon sample holder for SQUID and FMR measurements, respectively. X-band FMR spectra (Bruker ESP 300E) at 4 mW microwave power, with 2×10^4 receiver gain and 2.018 Oe field modulation amplitude and hysteresis curves (MPMS-XL Quantum Design SQUID magnetometer) were obtained at room temperature with the magnetic field applied parallel to the long body axis of the insect, as shown in Figure 1. The FMR absorption spectra areas (second integral of the derivative spectra) were calculated with a software developed using the graphic language LabVIEW®, starting at the high field values where the baseline is better defined.

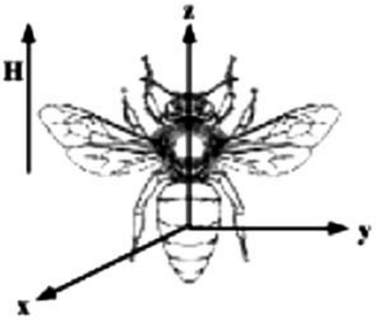

Figure 1. Insect orientation relative to the magnetic field.

Results

Hysteresis curves present a straight line with positive or negative slope at very strong fields due to paramagnetic or diamagnetic contributions, respectively. Bee, head, thorax and abdomen present a diamagnetic contribution (figure not shown), while

Table 1. Magnetic parameters of one *S. quadripunctata* bee[a] and body parts[b].

	Whole bee	Head	Antennae	Thorax	Abdomen
J_s (10^{-6} emu)	3.3 ± 0.4	1.1 ± 0.3	2.1 ± 0.3	0.7 ± 0.3	0.9 ± 0.5
H_c (Oe)	43 ± 15	32 ± 8	130 ± 5	44 ± 18	90 ± 20
J_r (10^{-7} emu)	2.0 ± 0.8	1.4 ± 0.4	5 ± 0.5	0.8 ± 0.1	0.8 ± 0.4
χ (10^{-9} emu/Oe)	−4.2 ± 0.5	−2 ± 0.2	+0.4 ± 0.1	−3.6 ± 0.2	−1.6 ± 0.2
J_r/J_s	0.06 ± 0.03	0.12 ± 0.06	0.24 ± 0.03	0.12 ± 0.03	0.09 ± 0.03
Magnetic (%)		23 ± 3	44 ± 4	15 ± 2	19 ± 4[c]
S (10^8 a. u.)		2.1 ± 0.1	5 ± 0.2	1.8 ± 0.1	1.7 ± 0.1
FMR (%)		20 ± 1	47 ± 3	16 ± 1	\16 ± 1

[a] Two bees average values.

[b] Four bees parts average values.

[c] Taking the control bee J_s value it increases to 30%.

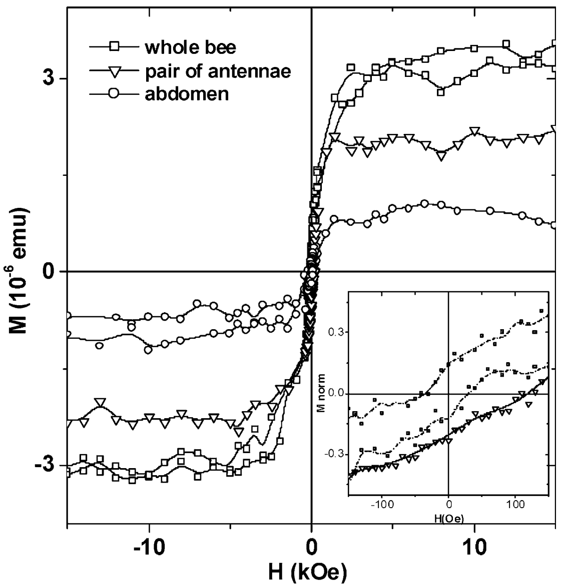

Figure 2. RT Hysteresis curves of *S. quadripunctata* whole bee, pair of antennae and abdomen, oriented parallel to the magnetic field, normalized to one individual and part. Insert: low field region of head (dashed line) and antennae (solid line) normalized hysteresis curves. Lines are guide to the eyes.

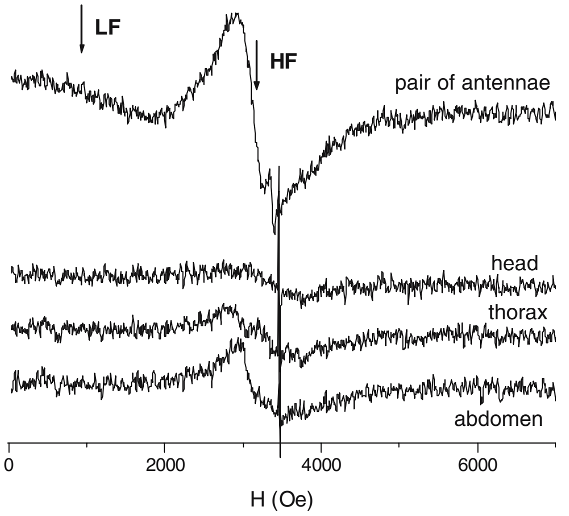

Figure 3. RT X-band ferromagnetic resonance spectra of *S. quadripunctata* body parts.

the antennae a paramagnetic one. The dia/paramagnetic susceptibilities (Table 1) are obtained by a linear fit of the curve at magnetic fields higher than that where ferromagnetic saturation is achieved and their contributions subtracted. Figure 2 presents the RT hysteresis curves normalized to one part and one individual, with the highest magnetic contribution coming from the antennae part. For clearness, thorax and head loops are not shown and only one branch of the abdomen and antennae loop were measured. The magnetic parameters: saturation magnetization, J_s, remanent magnetization, J_r and coercive field H_c, obtained for each body part and for one bee are given in Table 1, including the J_r/J_s ratio. The J_s sum of each body part average, $4.8 \pm 1.4 \times 10^{-6}$ emu, is taken to calculate the percentual contributions to J_s as 44 ± 4%, 23 ± 3%, 15 ± 2%, 19 ± 4% for antenna, head, thorax and abdomen, respectively. Considering the magnetic material differences content among individuals and the error bars, the total J_s is in good agreement with the average J_s of the two bees used as control.

The low field region of the head and antennae hysteresis curves in Figure 2, normalized to their J_s values, are given in the insert. The antennae present the highest H_c value (130 Oe) and J_r/J_s ratio (0.24), comparatively to the H_c (32–90 Oe) and J_r/J_s (0.09– 0.12) values of other parts. Considering magnetite as the magnetic particles material, the antennae particle sizes fall between 0.037 and 0.10 μm while the other body part particles are about 0.22 μm (Ozdemir *et al.* 2002).

Figure 3 shows the FMR spectra of the bee body parts with the magnetic field oriented parallel to the long body axis. Diamagnetism does not contribute to the FMR spectra while paramagnetism does and was not subtracted, as in the hysteresis curves. The four parts spectra present a broad (linewidth 550–900 Oe) component at high field, HF, centred at about 3000 Oe, with the antennae HF line intensity higher than the other ones. Only the antennae spectrum clearly presents another component at low field, LF, at about 1300 Oe. The values of the absorption areas S, (the second integral of the FMR derivative spectra) of the parts of the *S. quadripunctata* bee are given in Table 1. S calculated with the WINEPR (Bruker) software is not accurate when a component spreads out to zero field, as in the antennae case. The specially developed software used in this paper, corrects the assumption of zero intensity at the first spectrum field value by integrating from high to low field values. Even so, the antenna S value is a low limit value because the LF line is incomplete and the respective contribution cannot be fully calculated. S values of the HF at RT are related to the magnetic material amount, as shown by its linear relation to the saturation magnetization in termites (Oliveira *et al.* 2005). Correlation between integrated FMR intensity and the magnetization was also observed in Si doping of ferrihydrite nanoparticles (Seehra *et al.* 2001). Taking S as proportional to the number of resonant spins in the sample, the magnetic material percentages in each body part are: 47 ± 3%, 20 ± 1%, 16 ± 1% and 16 ± 1% in the antennae, head, thorax and abdomen, respectively. These values are in very good agreement with those above, obtained by SQUID magnetometry.

Discussion

Magnetoreception is a mechanism of magnetic field perception and transduction used for an organism's orientation. Two hypotheses have arisen to explain its basis: one considering biochemical reactions modulated by magnetic field, and another the presence of biogenic magnetic particles as magnetosensors. For now, much of what is known about this mechanism has been accumulated from behavioural experiments, theoretical proposals and a few electrophysiological and anatomical studies (Lohmann & Johnsen 2000). Recent results suggested the involvement of at least two types of receptors in obtaining magnetic compass information, with the specific interaction of these receptors being rather complex (Wiltschko *et al.* 2004). Biogenic magnetic particles have gained relevance as they have been reported in several species (Wiltschko & Wiltschko 1995; Safarik & Safarikova 2002), but their connections to nervous structures still need to be proved. Despite the difficulty of locating tiny magnetoreceptors, that might be dispersed anywhere within the animal body, FMR or SQUID magnetometry can be used to characterize their properties present in some social insects (Wajnberg *et al.* 2000; El-Jaick *et al.* 2001; Esquivel *et al.* 2002; Alves *et al.* 2004; Esquivel *et al.* 2004; Wajnberg *et al.* 2004; Oliveira *et al.* 2005a). In this paper, both techniques were used to study the body parts of *S. quadripunctata* bees. The HF and LF FMR components present in this bee body parts have already been observed in the abdomen of *A. mellifera* and *P. marginata* and associated to isolated and aggregated magnetite nanoparticles, respectively (Wajnberg *et al.* 2000; El-Jaick *et al.* 2001). Moreover, the relative amounts of magnetic material obtained from J_s and S strongly agree, confirming the usefulness of the latter in comparing amounts of magnetic materials at RT. The joint analysis of the magnetic material with both techniques in all body parts results as 23 ± 3%, 45 ± 5%, 15 ± 2% and 19 ± 4% magnetic material contributions of head, antennae, thorax and abdomen, respectively. It agrees on the stingless bee antennae containing the highest amount. As far as we know, this is the first study on magnetic material in all body parts of a honeybee other than *Apis mellifera*, the most studied one, besides optical and Electron Microscopy observations on *S. postica* abdomens (Cunha *et al.* 1987; Keim *et al.* 2002). A few previous FMR results (Takagi 1995; El-Jaick *et al.* 2001) confirmed the presence of ferromagnetic and paramagnetic material in *A. mellifera* abdomens, without measuring the other body parts. On the other hand, magnetic measurements of whole *A. mellifera* (Oliveira *et al.* 2005a), body parts (Takagi 1995) and particularly abdomens (Esquivel *et al.* 2002) have shown the presence of superparamagnetic and larger magnetic particles or aggregates in this body part.

Hysteresis parameters of whole honeybees and respective abdomens are compared in Table 2. Honeybees *A. mellifera* and *S. quadripunctata* present very different magnetic material properties,

Table 2. A. mellifera and *S. quadripunctata* magnetic parameters.

	S. quadripunctata	*A. mellifera*	*S. quadripunctata* abdomen	*A. mellifera* abdomen
J_s (10^{-6} emu)	3.3 ± 0.4	39 ± 4	0.9 ± 0.5	2.5
H_c (Oe)	43 ± 15	93 ± 10	90 ± 20	44
J_r (10^{-7} emu)	2.0 ± 0.8	46 ± 5	0.8 ± 0.4	2.4
χ (10^{-9} emu/Oe)	−4.2 ± 0.5	–	−1.6 ± 0.2	–
J_r/J_s	0.06 ± 0.03	0.11 ± 0.03	0.09 ± 0.03	0.09

except for the J_r/J_s ratio. The amount of magnetic material in *S. quadripunctata* is approximately 10 times lower than in *A. mellifera*, and almost three times lower in the abdomens as observed from the J_s values. For comparison, *A. mellifera* workers are about 12 mm long while *S. quadripunctata* about 6 mm, and the abdomens present the same length ratio.

The magnetic fraction present in the *S. quadripunctata* abdomen (19% Table 2) is higher than in *A. mellifera* (6%). Even considering the differences in magnetic material among individuals of the same species, this J_s fraction calculated based on the control bee J_s value (30%) evidences even more the honeybee differences. The estimated size of the particles in *S. quadripunctata* abdomens ($\sim$220 nm) is much larger than 13 nm of the *A. mellifera* estimated from FMR experiments. This difference can be related to: genus specificity, technique sensitivity (SQUID and FMR), sample preparations and environment conditions. The large size is in good agreement with 40–160 nm size range of the iron granules found in another stingless bee *S. postica* (Cunha *et al.* 1987), although ferritin-like granules were observed as electron-dense particles measuring 2.1 ± 0.5 nm in their abdomen (Keim *et al.* 2002). Stress should be given to the ingested magnetic material contribution in the thorax and abdomen, which is not biomineralized, and could be the cause of the different nanoparticle size and concentrations in abdomens. On the other hand, the head and antennae material can only be the result of a biomineralization process, which from an evolutionary point of view can produce a specific and efficient size and geometry. It is interesting to note that the *Pachycondyla marginata* ant, which migratory behaviour was related to the geomagnetic field (Acosta-Avalos *et al.* 2001), shows a similar result, with $42 \pm 3\%$ of the magnetic material in the antennae (Wajnberg *et al.* 2004). As far as we know, no experiments have been carried out concerning the antennae as a magnetoreceptor for orientation; however, the sensitivity of beetle and bug antennae to non-uniform microwave electromagnetic fields was studied, indicating that they can detect and respond to the radiation (Ondracek *et al.* 1976). Although no obvious organ or structure devoted to magnetoreception necessarily exists, bees possess complex sensory organs, as antennae and eyes, which should be considered. The antennae are composed of thousands of *sensilla*, which are connected to the central nervous system (Dade 1994). More than one decade ago, magnetite particles found in *A. mellifera* bee abdomens were suggested for magnetic orientation (Kirschvink & Walker 1985); nevertheless, the high fraction and size of this biomineralized magnetic material in the *S. quadripunctata* antennae led us to speculate that this part may be a magnetosensor organ. These preliminary findings should be corroborated with further behavioural studies and complementary physical characterization techniques to compare to other insect species, whose orientation behaviour is known to be influenced by the geomagnetic field.

Acknowledgements

We are grateful to R. Eizemberg for samples supply, Dr M. Castro for taxonomic information and to Dr O.C. Alves, Dr H.G.P. Lins de Barros for helpful discussion and Dr D. Guenzburger for carefully reading. MJL thanks CLAF-CNPq and EW thanks CNPq for financial support.

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