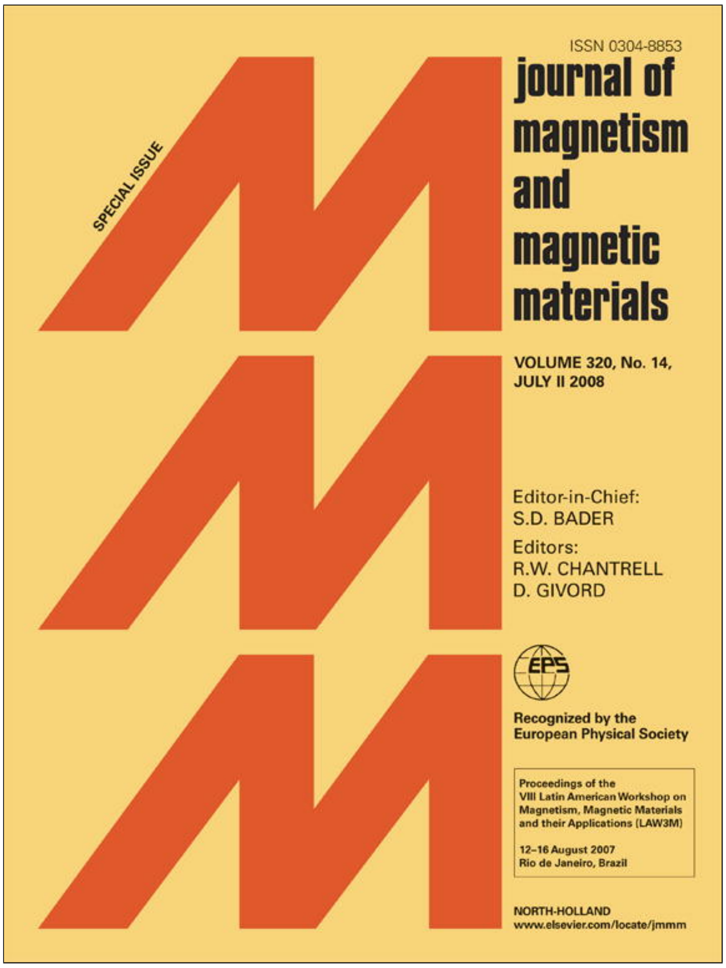
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Temporal and preparation effects in the magnetic nanoparticles of *Apis mellifera* body parts

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Abstract

Magnetic nanoparticles in the *Apis mellifera* abdomens are well accepted as involved in their magnetoreception mechanism. The effects of sample preparation on the time evolution of magnetic particles in the honeybee body parts (antennae, head, thorax and abdomen) were investigated by Ferromagnetic Resonance (FMR) at room temperature (RT), for about 100 days. Three preparations were tested: (a) washed with water (WT); (b) as (a), kept in glutaraldehyde 2.5% in 0.1 M cacodylate buffer (pH 7.4) for 24 h and washed with cacodylate buffer (C); (c) as (a), kept in glutaraldehyde 2.5% for 24 h and washed with glutaraldehyde 2.5% in cacodylate buffer (GLC). The four body parts of young and adult worker presented magnetic nanoparticles. The Mn^{2+} lines are observed except for the antennae spectra. The high field (HF) and low field (LF) components previously observed in the spectra of social insects, are confirmed in these spectra. The HF line is present in all spectra while the LF is easily observed in the spectra of the young bee and it appears as a baseline shift in spectra of some adult parts. The HF intensity of the abdomen is commonly one order of magnitude larger than any other body parts. This is the first systematic study on the conservation of magnetic material in all body parts of bees. The results show that the time evolution of the spectra depends on the body part, conserving solution and bee age. Further measurements are necessary to understand these effects and extend it to other social insects.

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Keywords: Magnetic material; Conservation; *Apis mellifera*; FMR

1. Introduction

The effect of the geomagnetic field on the *Apis mellifera* honeybee waggle dance was shown [1] and since this first result several other behavioral experiments were performed [2,3]. It is well accepted that magnetic nanoparticles in their abdomens are involved in magnetoreception, a still unknown mechanism. The ferromagnetic hypothesis, one of most acknowledged among those proposed to explain this mechanism, is based on the presence of these particles [4]. Magnetite nanoparticles of 30–35 nm diameters found in the bee abdomens [5] were assumed to be involved in magnetic field detection due to their stability. Later on, particles assumed to be of magnetite and with the dimensions about that of superparamagnetic (SPM) ones were observed in the second abdominal ganglion of bees [6]. Iron-containing trophocytes were found within the fat body of adult *A. mellifera* honeybee [7,8], identified as SPM magnetite particles [9]. These cells are innervated, which suggests that they might be primarily responsible for magnetoreception. It was proposed that these magnetite particles would act as an amplifier of the external magnetic induction changes, which would influence neuronal elements near those particles [6]. Recently, magnetic nanoparticles were found in other body parts of social insects, as ants [10,11], termites [12] and meliponinae bees [13]. The sample preparations of these papers were distinct one from each other and analysis of chemical and time effects on the magnetic material were not performed.

In this paper, the Ferromagnetic Resonance (FMR) technique is used to compare the effects of three different

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preparations on the room temperature (RT) spectra of young and adult honeybee's body parts. The spectral time dependence is studied during a period of about 100 days.

2. Material and methods

Apis mellifera honeybees were collected alive in Seropédica, Rio de Janeiro, Brazil. The bees were carefully washed with distilled water to clean the honey and impurities. They were separated in two groups, young (light colored) and adults (dark colored). Three preparations were performed: (a) washed with water (WT); (b) as (a), kept in glutaraldehyde 2.5% in 0.1 M cacodylate buffer (pH 7.4) for 24 h and washed with cacodylate buffer (C); (c) as (a), kept in glutaraldehyde 2.5% for 24 h and washed with glutaraldehyde 2.5% in cacodylate buffer (GLC). The bees were separated in four parts: antennae, head, thorax and abdomen. The samples were composed by seven pairs of antennae, five heads, three thoraxes and three abdomens. Young and adult worker body parts were studied for about 100 days.

Measurements were performed at RT, in a commercial 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, with the magnetic field applied perpendicular to the long body axis of the bees. The absorption curve area, *S*, which is proportional to the number of FMR resonant spins [11,14] can be used to estimate the magnetic material amount in insect body parts [11,13]. However, it could not be used in this work due to the complex line shape of *A. mellifera* spectra. The amplitude (I_{pp}) and the g factor of the spectral components were studied.

3. Results

The six Mn^{2+} hyperfine lines are observed except for the antennae spectra. A narrow line (linewidth ~14 Oe) at $g = 2$, Fig. 1. FMR spectra of *A. mellifera* body parts in WT, oriented perpendicular to the magnetic field at RT (a) young and (b) adult bees related to free radicals resulting from biological processes, is easily seen in all spectra (Fig. 1). These lines were previously observed in *A. mellifera* honeybees smashed abdomen [9].

Two ferromagnetic broad lines called high field (HF) and low field (LF) are present in the bee body parts spectra (Fig. 1). The spectral characteristics of these two components are different on each body part. The HF line g values are in the range from 2.05 to 2.20 with 400–500 Oe linewidths. The HF line is present in all spectra while the LF is more easily observed in the spectra of the young bee and in some spectra it appears as a baseline shift, with g values higher than 4.0 and linewidths from 500 to 1300 Oe. These lines were observed before in other social insects [9,11,15]. The HF intensity of both young and adult abdomens is commonly one order of magnitude larger than any other body parts.

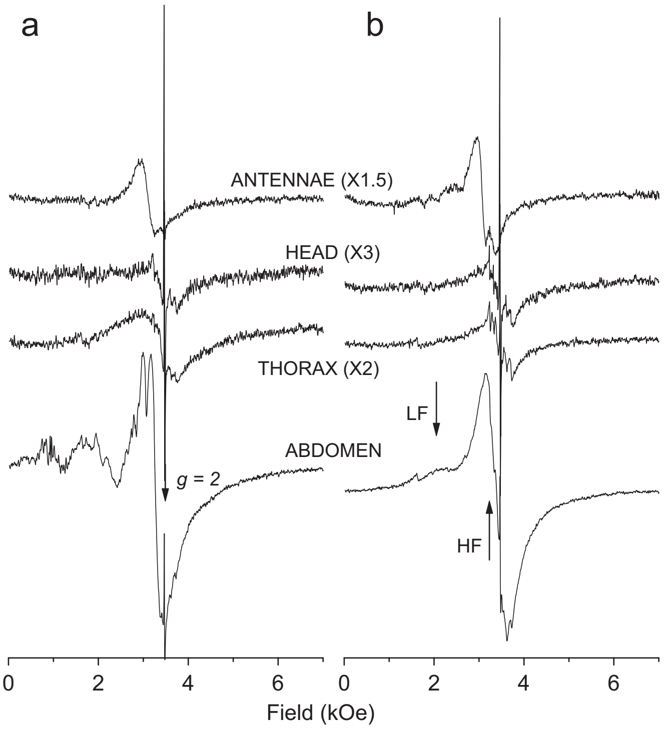

Fig. 1. FMR spectra of *A. mellifera* body parts in WT, oriented perpendicular to the magnetic field at RT (a) young and (b) adult bees.

Under a reliable association of the HF line with magnetite isolated nanoparticles [9,11,15] added to its possible biological function, this study was focused in this line. The change of the HF I_{pp} during the 100 days depends on the sample conditions as described. The HF I_{pp} of adult head samples in the three preparations and of the young head in WT decreases (Fig. 2a) while the I_{pp} of young head in GLC and C does not change.

The I_{pp} of adult thoraxes for any conservation condition decreases while I_{pp} of young ones does not change. The I_{pp} of young and adult abdomens in GLC and C first decrease followed by an increase while in WT the I_{pp} of adult abdomens increases and of the young does not change (Fig. 2b). The I_{pp} of adult antennae increases in C (Fig. 2c), decrease in WT and does not change in GLC, however the I_{pp} of young antennae increases in WT and GLC and it does not modify in C.

Comparing the spectra of young and adult body parts, the HF I_{pp} of the adult head is larger than the young one in WT but it is lower in C and in GLC, they are almost the same for both life stages. The young and adult thoraxes I_{pp} are almost the same in WT and GLC while the young I_{pp} is larger than the adult in C. The I_{pp} of adult abdomen is larger than the young ones for any conservation (Fig. 3a). The I_{pp} of young antennae in C is larger than the adult one (Fig. 3b) and it is the opposite in WT while the antennae I_{pp} in GLC are almost the same for both life stages.

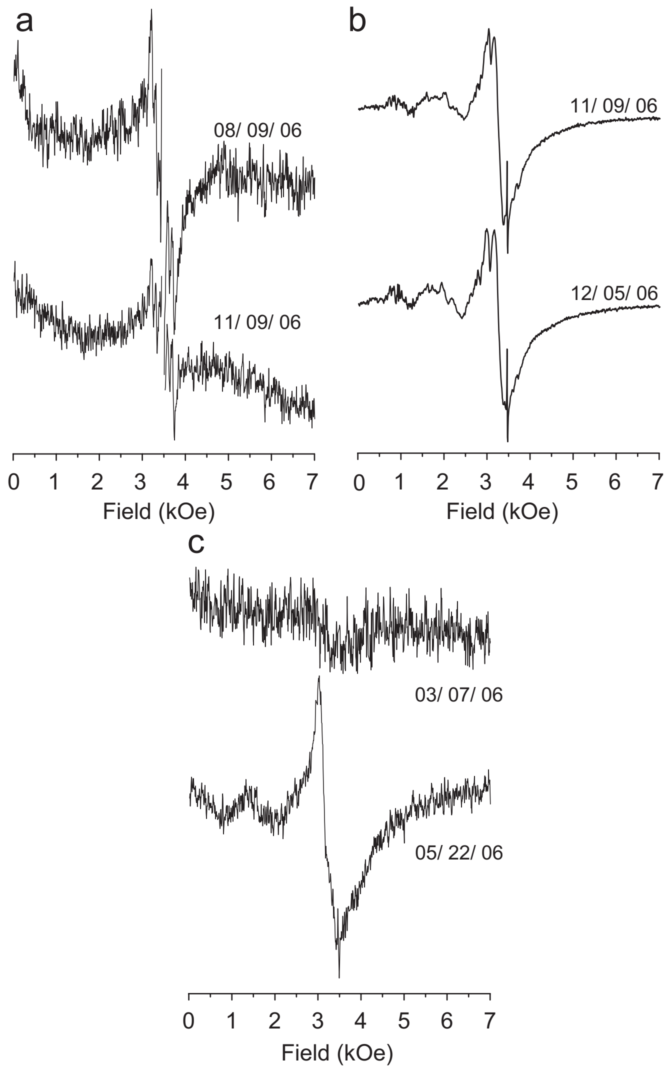

Fig. 2. Time dependence of FMR spectra (a) young head in WT, (b) young abdomen in WT and (c) adult antennae in C.

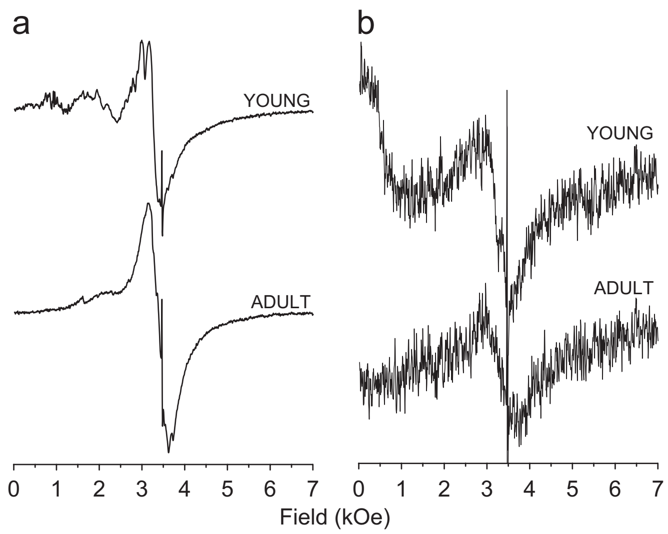

Fig. 3. FMR spectra of adult and young body parts at 12/05/06 (a) abdomen in WT and (b) antennae in C.

4. Conclusions

Previous SQUID magnetometry studies have suggested magnetite as the magnetic material in bee abdomens only [16,17]. The present FMR results revealed magnetic nanoparticles in the four body parts of young and adult honeybees. Although the HF spectral component showed the highest intensity, the LF component is also observed. These components have been associated to magnetite isolated nanoparticles (HF) and to large nanoparticles or aggregates (LF) in *A. mellifera* honeybee smashed abdomens [9] and other insects [11,15]. However, the low reproducibility of magnetic results have stimulated the analysis of time evolution and conserving solutions in these studies.

As far as we know, this is the first systematic study on conservation of magnetic material in all body parts of young and adult bees. The time evolution of the spectra depends on the conserving solution, body part and bee age. This subject is complex and some aspects should be considered. Although glutaraldehyde and cacodylate buffer are traditionally used for preserving magnetotactic micro-organism [18], there is not a unique conclusion regarding the preservation of the magnetic material in the bee body parts. The spectra time dependence of head and antennae can be different from the two other parts due to the presence of ingested material in thorax and abdomen. Likewise, the conservation solution can differently diffuse through each body part cuticle.

The higher LF relative to HF I_{pp} in young bees than in adult ones suggests changes of magnetic structures and points to the development of a magnetic sensor system during honeybees aging, which can be associated to their functions in the nest. Honeybees take repeated 'orientation' flights before becoming foragers at about 3 weeks of age [19] for which magnetoreception would be important. Otherwise, individual differences can be reflected in the results since the numbers of parts used in each sample cannot be a good statistic representation and changes in the natural arrangement of magnetic nanoparticles due sample preparation cannot be discarded. Further measurements are necessary to understand these effects and extend it to other social insects.

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