SHORT COMMUNICATIONS

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Evidence for Magnetic Material in the Fire Ant *Solenopsis* sp. by Electron Paramagnetic Resonance Measurements

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Electron paramagnetic resonance measurements were performed in smashed samples of fire ants, *Solenopsis* sp. The spectra are interpreted as a sum of different lines with different relative intensities. Results suggest the presence of magnetite particles of various sizes, reopening the discussion of the magnetic response in fire ants.

Adaptability of living beings to the environment includes complex navigation or orientation systems (Wehner 1996). In particular, successful mechanisms for search of food and homing are involved in the complexity of the foraging behavior in social insects, such as ants. These complex mechanisms are related to a variety of information sources, such as terrestrial landmarks (Wehner et al. 1996), the E-vector pattern of polarized light (Wehner 1992), the forest canopy (Oliveira and Hölldobler 1989), chemical cues (Hölldobler and Wilson 1990). For several years the geomagnetic field has been considered as another possible cue for orientation and homing in various organisms (Kirschvink et al. 1985). Change in the behavior of fire ants, *Solenopsis invicta*, due to artificial changes in the local geomagnetic field have been observed by Anderson and Vander Meer (1993). They proposed adding geomagnetic orientation as a mechanism that contributes to the foraging success of this species. However, recently Klotz et al. (1997) failed to replicate the experimental outcome of Anderson and Vander Meer, possibly due to discrepancies in the experimental set-ups, rendering a decisive comparison between the two studies impossible.

In the wood ant *Formica rufa* L., Çamlitepe and Stradling (1995) demonstrated a magnetic compass response using an appropriate experimental method suitable for testing magnetic compass response, leading to the conclusion that some ants may use the geomagnetic compass information for orientation. The discussion about the use of the geomagnetic field for navigation and orientation in fire ants is therefore still open. In summary, care must be taken in not considering failure to respond to a stimulus as a failure to perceive it (Wiltschko and Wiltschko 1996).

Geomagnetic sensitivity has often been associated with the presence of biomineralized material in general magnetite (Blakemore 1975; Kirschvink and Gould 1981; Kirschvink 1985). Slowik and Thorvilson (1996) reported the occurrence of subcuticular iron-containing tissue in fire ants, but they did not determine whether this iron was in a mineral form, or whether it was magnetic. Thus, evidence for the occurrence of magnetic material in ants, mainly magnetite, would contribute to reinforcing the magnetic orientation hypothesis.

Electron paramagnetic resonance (EPR) is a powerful technique to look for magnetic material without the need of its purification. EPR is a spectroscopic technique based on microwave absorption in the presence of an intense magnetic field, H. The absorbed microwave energy, given by $E = h\nu$, where h is the Planck's constant and ν is the microwave frequency, is given by the difference of the magnetic energy levels due to the permanent magnetic moment of the particle. In the condition of greatest simplification, the magnetic moments may be considered independent and noninteracting. Then the resonant magnetic field, H, is related to the microwave frequency, ν, by:

$$h\nu = g\beta H \qquad (1)$$

where g is the spectroscopic splitting factor and β is the Bohr magneton. With this technique, paramagnetic material can be characterized through its g factor value, and through its spectral characteristics in a first-derivative graphic of the absorbed microwave energy as a function of the applied magnetic field. The linewidth, ΔH, is the magnetic field difference between the points where the intensities of the absorption derivative signal are maximum and minimum. EPR is also a convenient technique for studying ferromagnetic materials, and in this case it is termed ferromagnetic resonance.

Because depending on the experimental condition a particular microwave frequency is used, a position in the spectra is normally given by the setup independent g_{eff} factor, instead of the resonant effective magnetic field value H', where g_{eff} is given by:

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$g_{eff} = h\nu / \beta H'$ (2)

To our knowledge, there is only one paper using this method in ant studies (Krebs and Benson 1965). That study, however, did not attempt to investigate ferromagnetic material. Here, for the first time, we present a study of magnetic material in fire ants using EPR spectroscopy.

Fire ants (*Solenopsis* sp.) from Citrolândia, Rio de Janeiro and Venda Nova, Espírito Santo, both in the Southeast Brazil, were used for the EPR experiments. Ants were collected at five different nest exit points in Citrolândia, probably from different nests. One of these nest exits was used three times on different days. Samples of Venda Nova were collected twice at the same place at an interval of 2 years. Smashed and desiccated samples of about a 100 ants each were transferred to sealed EPR tubes and followed by nitrogen flux. EPR was performed with an X-band (in the range of $\nu = 9$ GHz) spectrometer (Bruker ESP300 E) at room temperature. Experiments were performed on samples of different colonies. Experiments were carried out, and repeatedly checked, for several batches of the same colony. More than 50 spectra were obtained. Spectra from literature were digitised using a DaVinci, Digigraf digitizer after amplification. When necessary, Eq. 2 was used to infer magnetic field values from literature spectra. Frequency and field parameter corrections were taken into consideration for spectral comparisons, using the software Winspec. This software was developed specially for manipulation of a set of EPR data of different spectrometers and format.

Figures 1 and 2 show representative spectra of ant samples from Venda Nova and Citrolândia, Brazil, and spectra from literature, to be compared with ants' spectra, from natural mineral magnetite (Ikeya 1993) and fine and ultrafine magnetite particles (Aharoni and Morton 1971; Bandow and Kimura 1991).

The complex spectra of fire ants are composed of various lines, the presence and the intensity of these lines being dependent on the samples: ant origin, colony localization, etc. Moreover, spectra are dependent on the sample position in the cavity and on the orientation relative to the magnetic field.

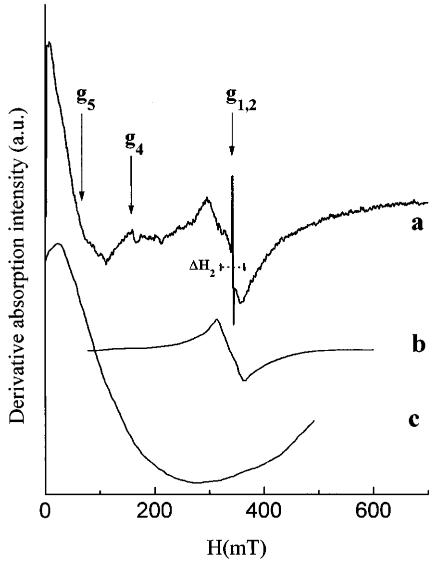

Fig. 1. EPR spectra showing the derivative of the absorption intensity (in arbitrary units) as a function of the applied magnetic field (in mT). *a*, EPR spectrum of fire ants from Venda Nova; *arrows*, resonance positions with their respective g_{eff} values: $g_{1,2} \approx 2.02$, $g_4 = 4.3$, and $g_5 = 12.5$. The linewidth $\Delta H_2 \approx 50$ mT is indicated for the absorption line at g_2. *b*, EPR spectrum obtained from magnetite particles (Aharoni and Morton 1971). *c*, EPR spectrum of natural mineral magnetite large particles (Ikeya 1993)

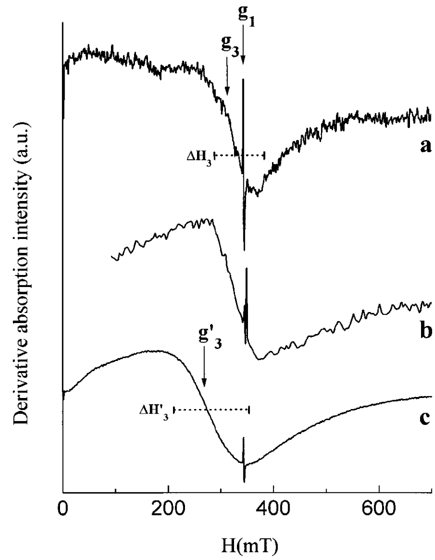

Fig. 2. EPR spectra showing the derivative of the absorption intensity (in arbitrary units) as a function of the applied magnetic field (in mT). *a,c*, EPR spectra of fire ants from Citrolândia; *arrows*, resonance positions with their respective g_{eff} values: $g_1 = 2.02$, $g_3 = 2.2$ and $g_3' = 2.62$. The linewidths $\Delta H_3 \approx 130$ mT and $\Delta H'_3 \approx 150$ mT are indicated for the absorption lines at g_3 and g'_3, respectively. *b* EPR spectrum of a magnetite film (Bandow and Kimura 1991)

In the region of $g \approx 2$, at least three lines are present: a very narrow at $g_1 = 2.02$, 1.8 mT linewidth, is observed in all spectra of ants (Figs. 1a, 2a,c). It is superimposed on another line with g value similar to the anterior one ($g_2 \approx g_1$) and $\Delta H_2 \approx 50$ mT, indicated in Fig. 1a. A wider one, with $g_3 \approx 2.2$ and $\Delta H_3 \approx 130$ mT, is predominant in the spectrum of Fig. 2a. The spectral features, g and ΔH, of those components and their relative intensities vary among the spectra obtained, as, for example, spectra of another sample of ants from Citrolândia (Fig. 2c) present a component that is wider and shifted to lower field values ($g'_3 \approx 2.62$, $\Delta H'_3 \approx 150$ mT), and in Venda Nova sample spectrum (Fig. 1a) the same line is narrower than the analogous one in Fig. 2a. Some spectra contain, in addition, a signal in the region $g_4 = 4.3$, shown in Fig. 1a, which is usually assigned to the presence of no aggregated Fe^{3+} in a site of low symmetry. A broad line spread from zero field up is observed in the spectrum of Fig. 1a with $g_5 = 12.5$.

Natural ferro (ferri)magnetic bulk materials can be distinguished by their EPR spectral features (Ikeya 1993). Otherwise complex spectra are observed in magnetite fine particle samples (Doorman et al. 1997, see references there). As biomineralized magnetic materials in living beings related to magnetoreception are generally identified as magnetite particles (Kirschvink 1985), the interpretation and identification of the spectra obtained from fire ants were based on previous EPR studies of mineral and synthetic magnetite. Thus, the spectrum of Fig. 1c from very large particles of natural mineral magnetite (Ikeya 1993) must be related to the zero-field component in Fig. 1a. On the other hand, small magnetite particles present EPR signals in the region of $g = 2$. A line at $g = 2.013$ (47.5 mT linewidth) was observed for samples of magnetite with average diameter of 3.5 nm (Aharoni and Morton 1971; Fig. 1b). This resembles the component with $\Delta H \approx 50$ mT in Fig. 1a. EPR measurements on ultrafine magnetite particle films show a wide ferromagnetic resonance observed at particle sizes larger than 3.4 nm, whereas a narrow resonance was related to the precursory state of the latter (Bandow and Kimura 1991). Figure 2b shows the spectrum obtained for 3.4 nm particles with the magnetic field parallel to the magnetic film ($g \approx 2.1$ and $\Delta H = 100$ mT), resembling the component with $\Delta H = 130$ mT in Fig. 2a. The central narrow line in spectra of ants can be due to either a precursory state or/and the presence of free radicals resulting from some biological processes.

In summary, the spectra obtained for ants strongly resemble those of magnetite. The spectra of fine particles are subjected to the dynamic effects of superparamagnetic system, and the position and linewidth of the EPR signal also depend on the particle volume distribution. Thus, the different signals in the $g = 2$ region could be associated with magnetic particles of different volumes. Spectra of ferromagnetic material can also be affected by the sample history caused by hysteresis effects. The observed dependence of the spectra on the orientation of the sample relative to the applied magnetic field is coherent with a partial orientation in a superparamagnetic system, resulting of the magnetocrystalline anisotropy of fine particles (Doorman et al. 1997).

The results indicate the presence of magnetic material in fire ants, and strongly suggest magnetite particles with various particle sizes. This type of magnetic material would enable magnetoperception based on the existence of single-domain and superparamagnetic magnetite particles, similar to those found in the honeybee (Gould et al. 1978; Kirschvink and Gould 1981; Schiff 1991). Whether fire ants actually use this capacity for orientation remains to be investigated. Perception and response are two different aspects of neural data processing. In *Solenopsis invicta*, as opposed to the honeybee and the wood ant *Formica rufa*, behavioral responses to magnetic stimuli are still controversial (compare Anderson and Vander Meer 1993; with Klotz et al. 1997). Indeed, behavioral studies on magnetic orientation, particularly in insects, are associated with several difficulties (see e.g., Wiltschko and Wiltschko 1996). Our findings will hopefully encourage further behavioral work aimed towards better understanding of ant orientation in natural environments.

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