

Definitive identification of magnetite nanoparticles in the abdomen of the honeybee *Apis mellifera*

M Desoil^{1,4}, P Gillis¹, Y Gossuin¹, Q A Pankhurst² and D Hautot^{2,3}

¹ Biological Physics Department, University of Mons-Hainaut, Belgium

² London Centre for Nanotechnology, University College London, London WC1E 7HN, UK

³ Institute for Science and Technology in Medicine, Keele University, Thornburrow Drive, Hartshill, Stoke-en-Trent, ST4 7QB, UK

E-mail: melanie.desoil@umh.ac.be

Abstract. The biogenic magnetic properties of the honeybee *Apis mellifera* were investigated with a view to understanding the bee's physiological response to magnetic fields. The magnetisations of bee abdomens on one hand, and heads and thoraxes on the other hand, were measured separately as functions of temperature and field. Both the antiferromagnetic responses of the ferrihydrite cores of the iron storage protein ferritin, and the ferrimagnetic responses of nanoscale magnetite (Fe_3O_4) particles, were observed. Relatively large magnetite particles (ca. 30 nm or more), capable of retaining a remanent magnetisation at room temperature, were found in the abdomens, but were absent in the heads and thoraxes. In both samples, more than 98 % of the iron atoms were due to ferritin.

1. Introduction

The honeybee *Apis mellifera* (figure 1) is known to be sensitive to magnetic fields. Its “waggle dance” is modified when the Earth's magnetic field is cancelled, which is a strong indication of a highly sensitive magnetic detection system.



Figure 1. The honeybee spends much time gathering food, as shown. The members of the bee-hive communicate information about the location of interesting food sources by the so-called “waggle dance”.

⁴ To whom any correspondence should be addressed.

Earlier measurements aimed at identifying their detection mechanism led to controversial conclusions. Gould et al. entitled their 1978 pioneering work “Bees have magnetic remanence” [1], and argued for the presence of magnetite particles about 30 nm in diameter, i.e., single domain particles, magnetically blocked at room temperature [2]. In 1986, agglomerates containing iron were discovered by Kuterbach and Walcott in bees’ abdomens, in trophocyte cells, a specific cell type of the fat body of insects [3]. Hsu and Li used transmission electron microscopy to identify those agglomerates as magnetite [4], with some isolated grains having a measured diameter of about 10 nm, corresponding to the superparamagnetic regime. This work was criticized by Nichol and Locke, and by Nesson, who suggested that the measurements had been misinterpreted due to a confusion of ferritin with magnetite; and also by Kirschvink and Walker, who questioned the chemical nature of the particles detected [5]. However, answering those criticisms, Hsu and Li maintained their point of view [5]. A precise characterization of the material involved in the bees’ magnetoreception system remains thus an open question.

2. Material and methods

Honeybees (*Apis mellifera*) were sacrificed by freezing, and then cut with plastic tools into two parts, on one side the head and the three thorax segments with the legs, and on the other side the abdomen. Samples were rinsed with distilled water, ground to a powder, and lyophilised for 24 hours. Magnetic measurements were made on a commercial SQUID magnetometer, following a recently improved experimental protocol [6]. Each sample comprised the tissue from the equivalent of ca. 15 freeze-dried bees.

3. Results and discussion

Magnetic data were recorded as functions of the applied magnetic field – hysteresis and isothermal remnant magnetisation (IRM) curves, and as functions of temperature – thermal decay of remanence (TDR). We present here the IRM data at 5 K and 50 K (figure 2), the TDR data (figure 3), and M-H curves (figure 4).

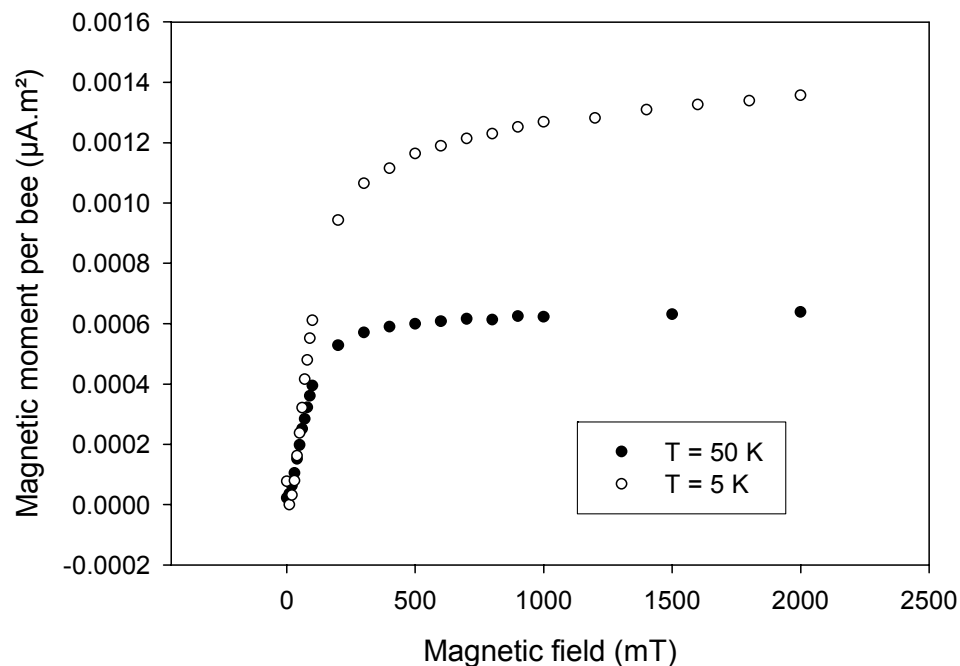


Figure 2. Remnant magnetisation of bees’ abdomens, measured in zero field after the application and removal of incrementally increasing magnetic fields, at 5 and 50 K.

Figure 2 shows that the magnetisation at 50 K, well above the blocking temperature of ferritin, saturates at ca. 400 mT. This is typical of magnetite nanograins (bulk magnetite would saturate for a slightly smaller field, ca. 250-300 mT), with a grain size that is larger than ca. 15 nm, the blocking temperature being specified by the equality $KV/(kT) = 25$, where K is the anisotropy constant, V the particle volume, k Boltzmann's constant, and T the temperature.

At 5 K the high field magnetisation approximately doubles, but does not saturate. The lack of saturation is most likely due to the contribution of the high coercivity antiferromagnetic ferritin. Using recently published data about ferritin [7] we estimate that there is ca. 60 times as much iron (by mass) in the form of ferrihydrite, inside the ferritin protein, than in the form of magnetite. That estimation rests on the assumption that the remnant ferritin magnetisation arises from uncompensated moments within the crystal with a contribution close to the saturation value, which results in a magnetisation ca. 5000 A/m, while the magnetite magnetisation is 480 000 A/m, and it accounts for the respective proportions of iron in both crystals (72 % in magnetite, 58 % in ferrihydrite).

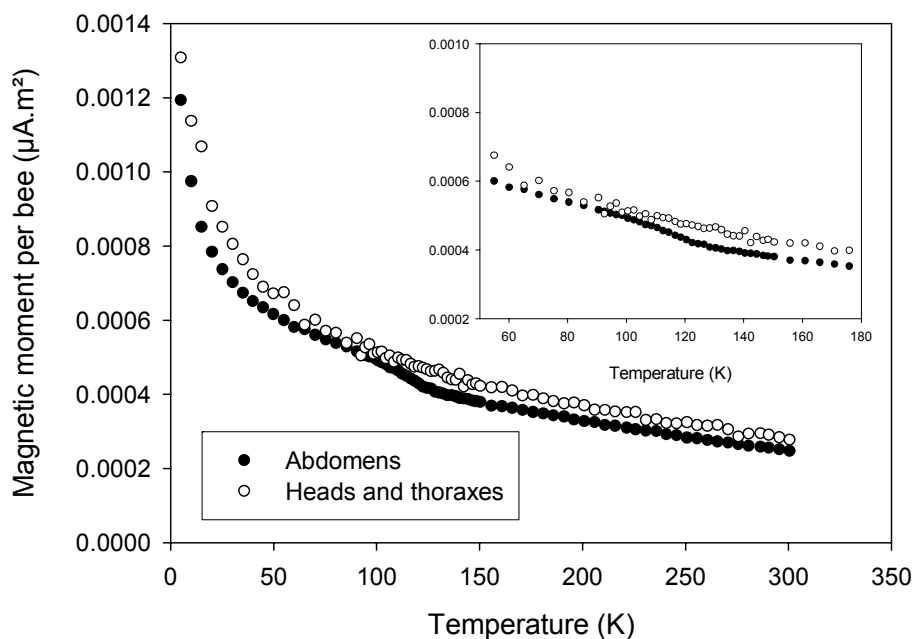


Figure 3. Thermal decay of remanence curves of bee's abdomens and bee's heads and thoraxes, measured upon warming in zero field after cooling from 300 K in an applied field of 2 T.

The bee's abdomen TDR data in figure 3 displays a shoulder, which could be assigned to the magnetite Verwey transition (enhanced in the insert) at ca. 120 K. The extended transition region, from ca. 110 to 130 K, is an indication of a distribution in the size of the magnetite particles, with the presence of relatively large particles, i.e., larger than 30 nm in diameter, since the Verwey transition was observed to vanish for smaller particles [8].

In contrast, no such shoulder is apparent in the bee's heads and thoraxes TDR curve. The Verwey transition is a signature of the presence of magnetite. We thus conclude, as near as further measurements confirm our "Verwey" interpretation, that "large" magnetite particles in honeybees are located in their abdomens, while smaller ones (with a size comparable with ferritin cores) may also be found in heads or thoraxes.

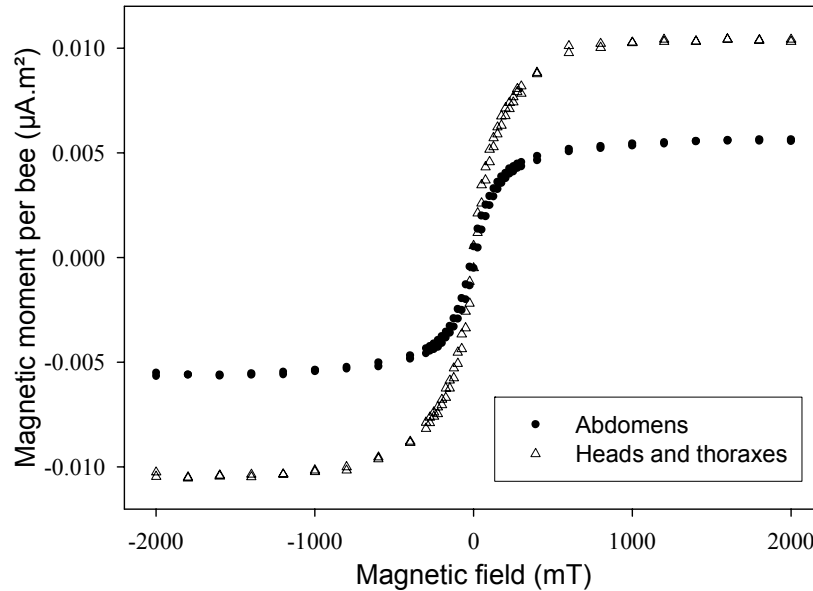


Figure 4. Hysteresis curves of bees' abdomens and bees' heads and thoraxes at 150 K, obtained after subtraction of the diamagnetic signal.

Figure 4 displays hysteresis in both signals, from the abdomens as well as from the heads and thoraxes. A temperature of 150 K is too high for the ferritin to exhibit a hysteretic behavior. The ratio of the remnant magnetisation over the saturation magnetisation is 0.09 for the abdomens and 0.05 for the heads and thoraxes; this is an indication that the magnetite particles present in the thoraxes are too small to produce a Verwey transition ($\langle d \rangle < 30$ nm) [8].

4. Conclusion

Elucidating the nature of magnetic sensors remains a fascinating challenge. The question of the size of the elementary “bricks” constituting the device plays a central role regarding that issue: small magnetite particles are superparamagnetic (SPM), larger ones are single domain, magnetically blocked at room temperature. Some researchers consider sensors using the properties of SPM particles, mainly mutual anisotropy induced by the dipolar coupling between nearby crystals [9]. However building sensors with single domain particles seems more natural, simply because an angular information is easier to get if the sensor has its own magnetisation direction, fixed with respect to the animal body, rather than a magnetisation aligned onto the external field. Our results provide valuable indications from that point of view, even if they still need to be braced by some other experimental data: bees' abdomens contain (relatively) large magnetite particles, with a size larger than 30 nm, while magnetite in heads and thoraxes is structured as smaller particles, unable to induce a Verwey transition. Finally, the quantitative domination of ferritin particles against magnetite particles in all organs encourage us to revisit the 1995 controversy [5] and to agree with the identification of the particles observed by electron microscopy as ferritin.

5. References

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