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Magnetite biomineralization in termites

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Experimental evidence exists for magnetoreception in termites, a major component of the soil macrofauna in many tropical countries. This preliminary study identifies, probably for the first time, the presence of biogenic ferrimagnets (magnetite?) in two species of termite (*Nasutitermes exitiosus* and *Amitermes meridionalis*), based on magnetic measurements of whole termite specimens and individual body sections, and analysis by electron microscopy of magnetically extracted grains. The magnetic measurements indicate the presence of very small concentrations of magnetic material, with more magnetic grains in the thorax and abdomen region compared with the head. Magnetic interaction, due to clustering of grains, is also identified by the measurements. Analysis of magnetic extracts by transmission electron microscopy identifies the presence of uniquely ultrafine (10 nm) and unidimensional grains of ferrimagnetic material, unequivocally distinct from any possible extraneous magnetite sources, such as ingested soil. Hence, this provides firm evidence for biogenic formation of this magnetic material by these two termite species. Such ultrafine grains would be superparamagnetic, i.e. incapable of carrying a permanent magnetic moment, unless they were sited in clusters of interacting grains, when some remanence-carrying ability—and hence magnetotaxis—would be possible.

Keywords: termites; biogenic magnetite; magnetoreception; magnetotaxis

1. INTRODUCTION

Rickli & Leuthold (1988) report experimental evidence for magnetoreception in termites. Returning foragers of the harvester termite, *Trinervitermes geminatus* (Termitidae), were diverted from their trail via a tube and then dropped through a trap door into the experimental arena. Here were arranged eight radial pheromone trails. The termites preferentially chose the trail closest to the nest direction, until magnetic North was artificially shifted by 33°, when the preferences also shifted accordingly. Magnetoreception in another social insect, the wood ant (*Formica rufa*), has also recently been demonstrated experimentally (Çamlitepe & Stradling 1995). These authors used a choice chamber and a surrounding solenoid to isolate, in the absence of all other orientational cues, a magnetic compass response by these insects. Previous analytical and behavioural studies have also identified the presence of biogenic magnetite, and its use as a magnetoreceptor, in honeybees (Gould *et al.* 1978). This paper reports an investigation of the possible presence of biogenic magnetite in two species of termite, *Nasutitermes exitiosus* and *Amitermes meridionalis* (Termitidae).

Biomineralization of strongly magnetic ferrimagnets, such as magnetite (Fe_3O_4), has been reported for an extremely diverse range of organisms, from single-celled bacteria (Blakemore 1982) to algae (Lins de Barros *et al.* 1981), honeybees (Gould *et al.* 1978), butterflies (MacFadden & Jones 1985), fish (including salmon (Kirschvink *et al.* 1985)), birds (especially homing pigeons (Walcott *et al.* 1979)) and mammals, including humans (Kirschvink 1981; Schultheiss-Grassi *et al.* 1997). Active formation of ferrimagnets by organisms has been ascribed direct ecological advantages, in terms of the possibility of geomagnetic-assisted motility (as, for example, in the case of magnetotactic bacteria), or navigation (as in salmon and homing pigeons). Two other possible biosensory responses from the presence of magnetite include its use as a relatively dense gravity receptor, enabling detection by organisms of 'way-up', and as a sensor of electric fields (Skiles 1985).

2. METHODS

A large number (thousands) of live *Nasutitermes* termites were collected from a mound at Norton Summit, in the Mount Lofty Ranges, South Australia. To exclude non-biogenic, soil-derived magnetite from the ensuing analyses, the termites were fed on pure cellulose for at least three days, to clear their guts of any ingested detrital material. The termites were washed in double-distilled water, again to ensure exclusion of extraneous mineral material. Two sets of experiments were then performed: (i) magnetic measurements, using a cryogenic magnetometer, of (10s of) intact specimens and also of separated body parts (i.e. of heads and of thoraces + abdomens); and (ii) magnetic extraction of mineral grains from ground termite tissues, and analysis of the extracts by transmission electron microscopy (TEM). In addition, a smaller number of *Amitermes* specimens (*ca.* 20) were obtained from A. Spain, CSIRO, Townsville, and individual intact specimens were subjected to magnetic measurements on the same cryogenic magnetometer.

To prepare individual body sections of *Nasutitermes* for the magnetic analyses, termites were freeze-dried (to immobilize any small magnetic particles, which might otherwise lose any induced magnetic orientation by Brownian motion within viscous cell media) and dissected using non-magnetic tools, especially a glass microtome blade. This sample preparation was kindly done by Ken Lee, CSIRO, Adelaide. For measurement of

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their magnetic remanence properties, the samples were placed on double-sided tape on the sample holder of cryogenic magnetometers at the Black Mountain Palaeomagnetic Laboratory, Canberra and the Geophysics Department, University of Edinburgh. For all measurements, the background signal from the sample holder and tape was subtracted from the sample values. To test for the presence of ferrimagnetic material within the body sections, the natural remanence of the body sections was first measured. To provide information on the mineralogy and possible size of any magnetic particles, the rate at which an artificial, applied magnetization (IRM) was acquired or lost in progressively increasing, aligning (DC) or randomizing (AF) fields was also measured (e.g. Thompson & Oldfield 1986). The maximum applied DC field was 500 milliTesla (mT; compared to the Earth's magnetic field of *ca.* 0.1 mT).

For the magnetic extraction of any constituent magnetic grains, washed and freeze-dried termites were ground in a tungsten carbide mill. The ground tissues were then dispersed in double-distilled water using an ultrasonic probe. Magnetic grains were extracted from the suspension with a rare-earth (cobalt-samarium) magnet, using the high field gradient at the edge of the magnet to concentrate even ultrafine (sub-micrometer) magnetic grains (Hounslow & Maher 1996). The extraction procedure was repeated until sufficient extract was obtained for analysis by TEM, using a Philips EM 400 at the Division of Soils, CSIRO, Adelaide. The resulting extract was dispersed in double-distilled water by ultrasonic means; drops of the dispersed suspension were then placed on carbon support grids and air-dried before examination under TEM.

3. RESULTS

(a) *Magnetic measurements*

For the *Nasutitermes* specimens, separate body sections ((i) heads and (ii) thoraces + abdomens) from individual specimens provided no measurable natural remanences, and IRM values barely measurable above the background signal of the sample holder (*ca.* 0.02×10^{-9} Am^2, where Am^2 is the magnetic moment). However, measurements of a sample of 12 head sections produced repeatable IRMs of 0.03×10^{-9} Am^2 and of 12 thoraces + abdomens, 0.1×10^{-9} Am^2. For the intact *Amitermes* specimens, IRM values varied between 0.2×10^{-9} and 13.98×10^{-9} Am^2.

For comparison, IRM values for individual head and thorax sections of monarch butterfly specimens (MacFadden & Jones 1985) were 0.43×10^{-9} to 1.66×10^{-9} Am^2. For honeybees, Kirschvink & Gould (1981) measured similar IRM values of 2×10^{-9} Am^2, finding most of the magnetic material in the front third of the abdomen. Figure 1 plots the curves of IRM (in progressively increasing DC fields) with the AF demagnetization of the IRM (in progressively increasing alternating fields). For magnetoreception purposes, the most efficient grain size for a magnetic particle to act as a permanent magnet (i.e. like a compass) is *ca.* 0.03–0.05 μm in magnetite (Dunlop 1973; Maher 1988). Such particles are termed stable single-domain grains; they are composed of a single magnetic domain whose magnetic moments are uniformly aligned. They will act as a compass needle, swinging into alignment with the geomagnetic field. Particles smaller than single-domain size (less than *ca.* 0.03 μm) carry no permanent magnetic moment as they are subject to thermal randomization at

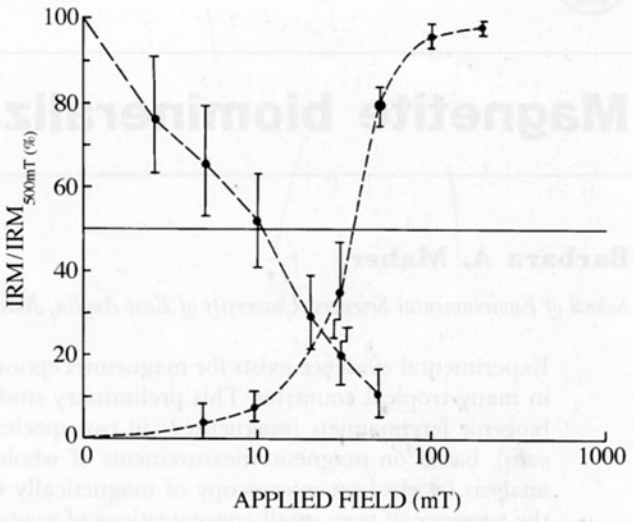

Figure 1. DC magnetization and AF demagnetization curves for *Amitermes* specimens (error bars = ± 1 s.d.).

room temperature; such particles are termed 'superparamagnetic'. Particles larger than this tend to subdivide internally into two or more magnetic sub-regions (i.e. multidomain grains), with opposing alignments; this reduces both their spontaneous and remanent magnetization. Single-domain magnetite acquires its magnetization over a restricted range of applied fields, normally between *ca.* 20 and 300 mT. Magnetization acquired below *ca.* 20 mT suggests either the presence of larger, multidomain grains or grains near the superparamagnetic/single-domain boundary. Magnetization acquired in fields above 300 mT identifies the presence of other magnetic minerals, such as goethite and/or haematite.

The IRM acquisition and demagnetization curves (figure 1) show some scatter but most magnetization (>80%) is acquired between 10 and 300 mT and most is demagnetized between 10 and 50 mT. Minor amounts of remanence are acquired both at lower and higher fields. In the presence of well-dispersed, single-domain magnetite particles, the acquisition and demagnetization curves would be mirror images of each other around the 50% magnetization point (Cisowski 1981). However, as seen in figure 1, there is considerable offset in the termite magnetic data; this suggests that there is significant magnetic interaction between particles (such interaction acting to inhibit magnetization and assist demagnetization).

(b) *Electron microscopy of magnetic extracts*

Using the magnetic edge extraction method, a very small amount of black, magnetic material was extracted from the termite tissues. TEM was used to identify the size and shape of the extracted particles. Clusters of ultrafine, electron-dense, iron-rich crystals were dominantly found in the extract, the clustering reflecting the magnetic nature of the crystals (figures 2 and 3). Most notable, however, is the extremely narrow range of grain sizes shown by the termite particles. The crystals average 10 nm in diameter. Despite their ultrafine size, the crystals display generally good crystallinity, with clear crystal faces seen around hexagonal and cubic morphologies (figure 3). The hexagonal crystal morphology is reported to be unique to biogenic forms of magnetite (Towe & Moench 1981).

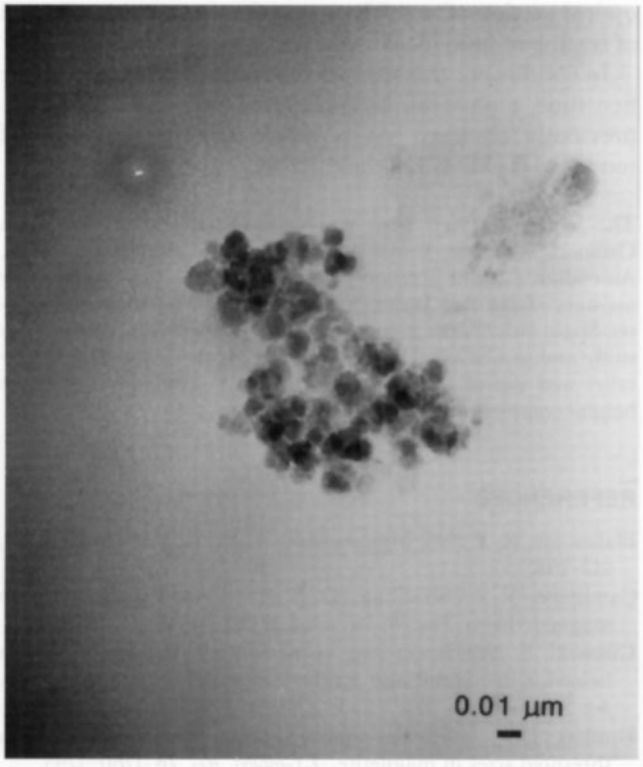

Figure 2. Transmission electron micrograph of black magnetic grains extracted from crushed termite tissues.

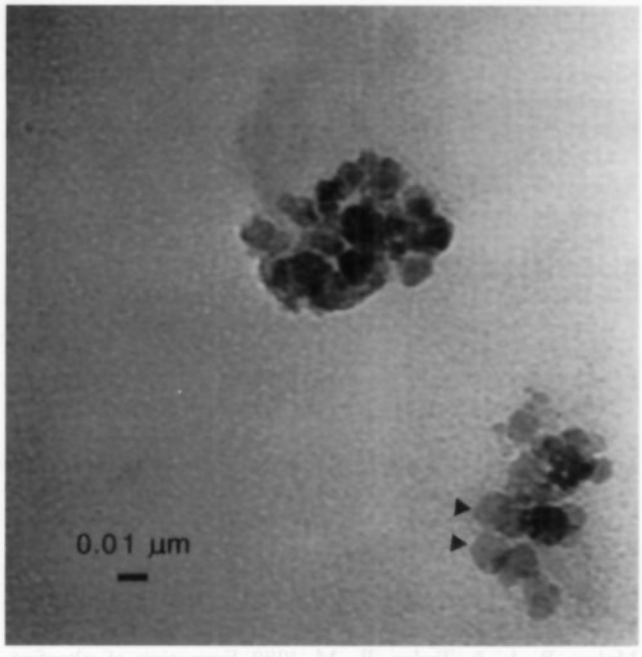

Figure 3. Transmission electron micrograph of termite magnetic extract; note the unidimensional nature of the grains and their distinct crystal faces and morphologies (e.g. marked by arrow).

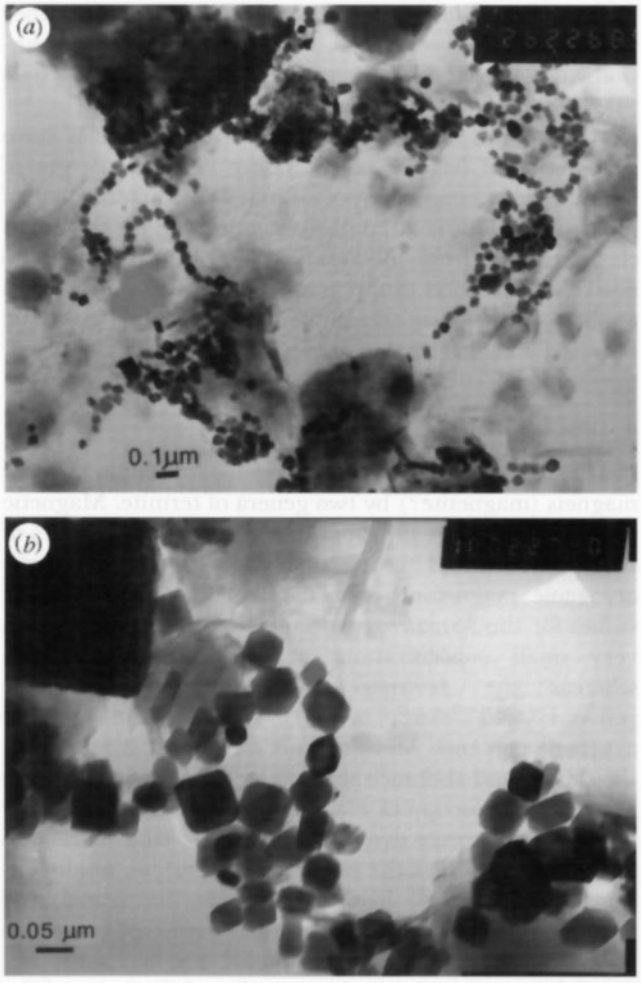

Figure 4. (*a*, *b*) Transmission electron micrographs of magnetite particles formed intracellularly by magnetotactic bacteria (magnetic extract from deep-sea sediment).

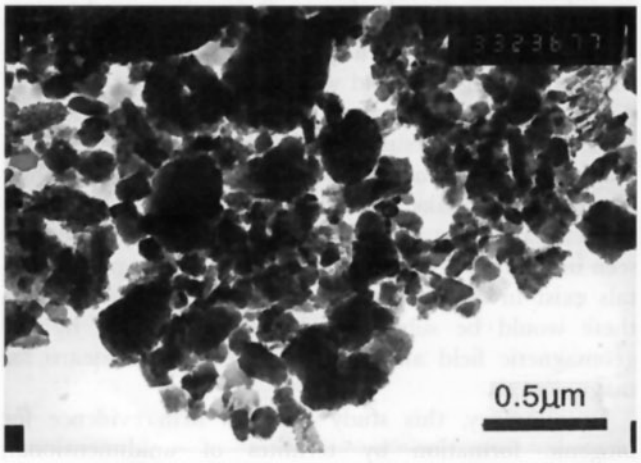

Figure 5. Transmission electron micrograph of magnetite particles formed extracellularly within the soil matrix (magnetic extract from a rendzina soil profile, Broadway, Cotswolds, UK).

For comparison, figures 4*a*,*b* and 5 show ultrafine magnetite particles extracted from magnetotactic bacteria and a soil sample, respectively. As can be seen from figure 4*a*,*b*, magnetic crystals formed biogenically are normally unidimensional owing to their intracellular origin. Their crystallization is controlled by the size and shape of the organic membrane that surrounds them (and into which the magnetite is precipitated). Compared with the termite magnetic grains, the bacterial magnetite crystals dominantly fall within a larger, single-domain grain size (*ca.* 0.03–0.05 μm). The soil magnetites display some grains as small as those from the termite tissues, but, in contrast, they vary markedly in their grain size. This reflects their extracellular origin, with unconstrained precipitation within the soil matrix (Maher &

Taylor 1988). The resulting crystals demonstrate a rather wide grain-size distribution, reflecting the rate of formation and mutual interference between growing crystals (Taylor *et al.* 1987).

Other iron-rich, ultrafine (*ca.* 7.5 nm) particles have been reported (e.g. Nichol & Locke 1995) in the gut of many insects, consisting not of magnetic material but of holoferritin. This is an unlikely composition for the particles extracted from the termites; holoferritin would be unlikely to respond to the magnetic extraction procedure applied to the termite samples, or show the magnetic aggregation of grains seen under TEM.

4. DISCUSSION

This investigation identifies biogenic formation of ferrimagnets (magnetite?) by two genera of termite. Magnetic measurements were difficult due to the low IRM values of the specimens and the (relatively) low sensitivity of the cryogenic magnetometer available. However, the IRM values for the *Nasutitermes* specimens suggest they contain very small concentrations of magnetizable material, whereas the *Amitermes* values, although variable (s.d. $= \pm 4 \times 10^{-9}$ Am^2, $n = 19$), suggest some higher magnetic contents. More magnetic material is present in the thorax and abdomen sections rather than in the head. Electron microscopy of black magnetic grains, extracted from ground termite tissues by a magnetic-edge extraction procedure, identifies the presence of ultrafine, unidimensional, well-formed, iron-rich crystals, hexagonal and cubic in outline. The unidimensional nature of the grains strongly suggests an intracellular origin, with cell membranes constraining the growth and final size of the precipitating crystals. Given the size of the crystals (*ca.* 0.01 μm), well below the single-domain grain size boundary in magnetite, their magnetic behaviour would be expected to be superparamagnetic (i.e. incapable of carrying a permanent magnetization at room temperature). However, the actual behaviour of such grains in the Earth's magnetic field will depend on their degree of dispersion or interaction. Clustering of superparamagnetic grains can result in collective single-domain-like magnetic behaviour, with some remanence-carrying capability (Radhakrishnamurthy *et al.* 1973; Maher 1988). If, as indicated by the offset in the magnetization curves seen in figure 1, a number of the superparamagnetic crystals exist in termite tissues as interacting clusters, then these would be subjected to magnetic torque by the geomagnetic field and could thus provide a means for magnetotaxis.

In summary, this study provides firm evidence for biogenic formation by termites of unidimensional, ultrafine-grained magnetic material; the material magnetically extracted from crushed termite tissues is unequivocally distinct from possible extraneous sources, such as ingested soil. Direct detection of magnetite *in situ*, to determine any association with magnetoreceptor cells, is difficult owing to the extremely small size of the magnetic grains and their very low volume concentrations in tissues, a 'needle-in-the-haystack operation' (Kirschvink 1997). However, new microscopy techniques (currently being developed by a multidisciplinary research team (Walker *et al.* 1997)), provide the prospect both of identification in optical section of possible magnetite crystals in cells and of tracing of individual associated neurons.

In conclusion, this study demonstrates, probably for the first time, a physical, biogenic basis for the magnetotaxis previously observed via a behavioural investigation of termites (Rickli & Leuthold 1988).

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