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Magnetic resonance imaging in entomology: a critical review

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Abstract

Magnetic resonance imaging (MRI) enables *in vivo* imaging of organisms. The recent development of the magnetic resonance microscope (MRM) has enabled organisms within the size range of many insects to be imaged. Here, we introduce the principles of MRI and MRM and review their use in entomology. We show that MRM has been successfully applied in studies of parasitology, development, metabolism, biomagnetism and morphology, and the advantages and disadvantages relative to other imaging techniques are discussed. In addition, we illustrate the images that can be obtained using MRM. We conclude that although MRM has significant potential, further improvements to the technique are still desirable if it is to become a mainstream imaging technology in entomology.

Keywords: Magnetic resonance microscopy, MRI, MRM, *Vespula vulgaris*, *Dinoponera quadriceps*

Abbreviation:

CSI	chemical shift imaging. The dependence of the resonance frequency of a nucleus on the chemical binding of the atom or molecule in which it is contained.
(N)MRI	(nuclear) magnetic resonance imaging
MRM	magnetic resonance microscopy
Voxel	A contraction for volume element, which is the basic unit of MR reconstruction; represented as a pixel in the display of the MR image.

This paper includes three videos that can be accessed at http://insectscience.org/3.5

Introduction

Nuclear magnetic resonance imaging (NMRI), usually shortened to magnetic resonance imaging, (MRI), is a non-invasive internal imaging technique used extensively in clinical diagnosis and medical research (Wehrli *et al*., 1988; Callaghan, 1991). As well as its ability to capture high quality *in vitro* images, MRI can be performed *in vivo* without harmful effects (Callaghan, 1991). Here we introduce the principles of magnetic resonance imaging and review its use in entomology. We illustrate the types of images that can be obtained from entomological material both *in vitro* and *in vivo*, and we discuss the advantages and disadvantages of MRI over conventional imaging techniques.

Magnetic resonance imaging generally utilizes interactions between protons (^{1}H) in liquid phase molecules (e.g. water and lipids) and a magnetic fields. These arise because the protons have a weak magnetic moment, and so behave like tiny magnets. The sample is placed within the bore of a powerful, but biologically harmless, magnet (typically producing a field of 0.5 – 2 Tesla, although magnets of up to 17 Tesla strength may be used; as a reference, the Earth's magnetic field is ca. 5 x 10^{-5} Tesla) and radio-frequency pulses are applied to it. The radio-frequency pulses that must have the appropriate resonant frequency known as the Larmor frequency, interact with protons in the sample, causing them to absorb energy and jump to an excited state. As a result, the protons in the sample coherently precess about the applied magnetic field and generate radio waves at the Larmor frequency. This radio-frequency emission is picked up by a receiver coil and the resulting NMR signal is used as the basis for imaging. The NMR signal changes over time depending on the protons' local microenvironment. For example, protons in fats have a different microenvironment than those in water, and thus produce a signal of different frequency. The differences in NMR signals produced by different tissues provide contrast in the image produced. Contrast can also be manipulated by changing the radio-frequency pulse sequence parameters, principally the repetition time and the echo time. In addition to protons, nuclei such as ^{13}C

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and ^{31}P, also exhibit magnetic resonance and may be used as the basis for imaging.

By applying three orthogonal magnetic field gradients, it is possible to determine the resonance signal from individual volume elements, known as voxels, within the sample. Computer integration and transformation of the signals received from the sample allows a two-dimensional map of proton density to be constructed, which can be visualized as a virtual "slice" through the sample. The resolution of the image is determined by the size of the voxels. Multiple two-dimensional slices through a sample can also be acquired in different ways to provide a three-dimensional image.

Examples of Entomological Use of MRI and MRM

Although MRI is commonplace in clinical situations, conventional medical imaging systems generally have insufficient resolution for entomological studies (Goodman *et al*., 1995). It was only with the development of a high-resolution version of MRI, known as magnetic resonance microscopy (MRM), that the high-resolution, non-invasive imaging of small organisms and even cells (Aguayo *et al*., 1986) became possible (Eccles and Callaghan, 1986; Johnson *et al*., 1986). Recent developments in MRM have seen resolution increase from a voxel size of 10 µm x 13 µm x 250 µm (the latter being slice thickness) for the ova of the African clawed frog, *Xenopus laevis* (Aguayo *et al*., 1986) to 1 µm x 1 µm x 75 µm for an inert sample, and 2 µm x 2 µm x 50 µm for a geranium stem (Lee *et al*., 2001). Because of practical limits imposed by magnetic field strength and imaging time, it is thought that 1 µm is the current limit to MRM resolution (Glover and Mansfield, 2002).

MRM in entomological parasitology studies

Detection of parasites within an insect provides a good example of the use of MRM. Conventional endoparasitoid studies typically use invasive dissection techniques in conjunction with light microscopy. Such studies are limited in their ability to provide good evidence on the behavior and spatial distribution of parasitoids within insect hosts. Additionally, conventional approaches are destructive and therefore cannot repeatedly sample a given individual (Chudek *et al*., 1998). Consequently, many individuals are needed to represent the full developmental sequence of the parasitoid. The need for many different individuals makes inter-individual variation in both parasitoid and host a serious confounding problem (Chudek *et al*., 1996; Mapelli *et al*., 1997). Additionally, multiple sampling may be a problem with specimens that are rare or difficult to obtain.

Non-invasive, and therefore non-destructive, MRM allows parasitoids to be identified to a useful taxonomic level and their development and behavior visualised and investigated in live hosts throughout the infection period (Thompson, 1991). Successful applications include the imaging of development, behavior, and host damage done by ichneumonid and braconid parasitoids of the Indian meal moth and the seven-spot ladybird beetle (Chudek et al., 1996; Chudek et al., 1998; Geoghegan et al., 2000; Table 1a). The ability to integrate successive two-dimensional image "slices" of a sample into a three-dimensional image enables the precise spatial relationships between parasitoid and host to be evaluated, which is not always possible with conventional techniques. In addition, it has been suggested that the prevalence of parasitoid infection within a population could be determined non-invasively using MRM (Chudek *et al*., 1998).

MRI in entomological developmental studies

Insect development, particularly that of holometabolous insects, provides significant challenges for the developmental biologist. As with parasitology, MRM enables the metamorphic process in a single organism to be imaged throughout its development reducing the confounding effects of inter-individual variation. Indeed, the first use of MRI in entomology was to image the embryonic development of a locust (Gassner *et al*., 1987, Table 1b). One particular technique, termed 'chemical shift imaging' (see below), enables the differentiation of lipids and water and allows their distribution to be determined separately (Goodman *et al*., 1995). In this way, Goodman et al. (1995) were able to use MRM to follow the use of food reserves by *Graphiphora augur* moths throughout their development (Table 1b). It has also been possible to image the changes in relatively small structures within the developing imago, such as silk glands in *Bombyx mori* (Mapelli *et al*, 1997; Table 1b). Other entomological developmental studies that have used magnetic resonance microscopy are reviewed in Table 1b.

Chemical shift imaging in pH metabolism studies using MRI

MRI mostly exploits the magnetic resonance properties of protons to build up internal images of samples. However, other nuclei such as ^{13}C and ^{31}P, also exhibit magnetic resonance and these too can be used for imaging. Imaging using ^{31}P has one important advantage over proton imaging. Moon and Richards (1973) showed that the chemical shift (the variation of the resonance frequency of a nucleus because of its magnetic environment) of every organic phosphate compound is dependent on pH. Consequently, it is possible to use chemical shift imaging (CSI) to map, and therefore effectively "image", pH *in vivo*.

Skibbe *et* al. (1995a) used CSI to provide pH maps of the midgut of the larva of *Spodoptera litura,* the tropical armyworm (Table 1c). Typical resolutions achieved in ^{31}P medical MRI correspond to pixels or voxels of edge dimensions ranging from 3.1mm to 2.5cm (Hugg *et al.*, 1992). However, Skibbe *et al*. (1995a) were able to achieve sub-millimeter resolution (pixel size of 0.625mm) and thereby perform high-resolution pH mapping at a scale suitable for entomological studies. Furthermore, by varying the feeding characteristics of individual insects they were able to provide "significant and useful biological information" on pH metabolism. The non-invasive aspect of MRI is again central to this application.

Traditional techniques to image pH inside cells or tissues use pH-sensitive fluorescent dyes (Mason, 1999) or microelectrodes for the targeting of specific tissues inside an organism (e.g. Romero et al., 1997). However, pH-sensitive dyes, can only be used to image pH in cell cultures (Mason, 1999), and only few insect cell lines can currently be cultured. In addition, the insect cuticle would be hard to penetrate by a microelectrode. Clearly then, CSI has considerable potential in entomological studies.

MRI in biomagnetism studies

Natural internal magnetism affects magnetic resonance images, producing characteristic distortion patterns. Consequently,

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(a) Parasitology

Host	Parasite	Study details	Reference
Periplaneta fuliginosa (Smoky-brown cockroach, Blatteria: Blattellidae)	Cockroach densovirus	Imaging of infected hosts	Takahashi et al., 1989
Ploidia interpunctella (Indian meal moth, Lepidoptera: Pyralidae)	*Venturia canescens* (Hymenoptera: Ichneumonidae)	Imaging of parasitoid development and behaviour Imaging of host tissue degradation	Chudek et al., 1996
Coccinella septempunctata (7-spot ladybird, Coleoptera: Coccinellidae)	*Dinocampus coccinellae* (Hymenoptera: Braconidae)	Imaging of parasitoid larvae Identification of parasitoid in host Imaging of host tissue degradation	Chudek et al., 1998 Geoghegan et al., 2000

(b) Development

Organism	Study details	Reference
Schistocerca gregaria (African migratory locust, Orthoptera: Acrididae)	Embyogenesis by imaging of eggs from just after fertilisation to a few days before hatching	Gassner & Lohman, 1987 Lohman & Gassner, 1987
Manduca sexta (Tobacco hornworm, Lepidoptera: Sphingidae)	Imaging of the developing caterpillar to a resolution of 100 µm Detection of motion using MRM	Conner et al., 1988
Pieris brassicae (Cabbage butterfly, Lepidoptera: Pieridae)	Imaging of pupal development Separate determination of waster and li pid distribution b y	Goodman et al., 1995
Graphiphora augur (Double dart, Lepidoptera: Noctuidae)	Imaging of pupal development Imaging of or gan systems	Goodman et al., 1995
Spodoptera litura (Tropical armyworm, Lepidoptera: Noctuidae)	Imaging of larvae for technological development purposes	Skibbe et al, 1995b
Bombyx mori (Silkworm, Lepidoptera, Bombicidae)	Imaging of larvae and pupae during postembyonal metamorphosis Imaging and characterisation of silk glands during metamorphosis	Mapelli et al., 1997
Sarcophaga peregrina (Flesh fly, Diptera: Sarcophagidae)	Imaging of pre-pupal and pupal development Development of ima ginal tissues	Price et al., 1999

(c) Metabolism

Organism	Study details	Reference
Spodoptera litura (tropical armyworm, Lepidoptera, Noctuidae)	Chemical shift imaging of pH metabolism in the midgut	Skibbe et al., 1995a

(d) Biomagnetism

Organism	Study details	Reference
Solenopsis invicta (fire ant, Hymenoptera, Formicidae)	Imaging of ferromagnetic substances in the heads of workers, queens and males, possibly used in orientation behaviour.	Slowik et al., 1997

(e) General morphology

Organism	Study details	Reference
Apis mellifera (honey bee, Hymenoptera, Apidae)	Imaging of internal structures of queens and drones. Ovary, crop, midgut, spermatheca, median oviduct, stin g cavity, rectum and air	Tomanek et al., 1996
Dinoponera quadriceps (Hymenoptera, Formicidae)	Imaging of the internal structures of an ant. The digestive tract is clearly visible.	Fresneau et al., 1991
Drosophila melanogaster (fruit fly, Diptera, Drosophilidae)	Imaging, but images are poorly resolved.	Fresneau et al., 1991
Pachycondyla apicalis, Dinoponera australis and *D. quadriceps* (Hymenoptera, Formicidae)	Imaging of ants kept in cooling chamber, but organs poorly resolved because of organ movements and muscle contractions. *In vitro* imaging yields better resolution; e.g. the ovaries are clearly visible.	Struyf, 1997
Sarcophaga bullata (blowfly, Diptera, Sarcophagidae)	Imaging of the brains of wax-immobilized blowflies. Excellent resolution but increased post-imaging mortality.	Jasanoff & Sun, 2002
Dytiscus marginalis (diving beetle, Coleoptera, Dytiscidae)	In vitro imaging to attain a spatial resolution of 30µm in less than 1 hour	Wecker et al., 2002

Table 1. Review of the use of magnetic resonance imaging microscopy (MRM) in entomology. Studies are *in vivo*, unless stated otherwise.

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it is possible to assay entomological material for natural magnetism using MRI, and to characterise and locate the source of magnetism within an insect's body. Slowik *et al*. (1997) analysed fire ant (*Solenopsis invicta*) workers, queens and alates for the presence of natural magnetism using magnetic resonance imaging (Table 1d). The halo-like bipolar ring patterns they observed indicated the presence of small amounts of internal ferromagnetic material in the head, which may be used in orientation behaviours.

MRM in general entomological imaging studies

While many entomological studies involving MRI have had a well defined motive for using the technique as discussed above (e.g. to study parasitology, developmental or pH metabolism) other studies have applied MRI more generally, with the intention of investigating how effective MRI is at producing internal images (both *in vitro* and *in vivo*) of various entomological samples.

In a study of the honey bee (*Apis mellifera*), Tomanek *et al.* (1996) acquired MRM images of the internal structure of drones (males) and queen honey bees. They present sagittal and transverse cross-sections (thickness 440 µm thick, with voxel size of 30 µm x 30 µm) through the head, trunk and abdomen of a drone and a sagittal cross-section through the abdomen of a queen. They used the sagittal cross-sections of the drone as a 'pilot view' of the insect in order to position the transverse cross-sections in areas of interest. As a strategy to target key structures of interest, this technique considerably reduces the image acquisition time since only one or a few slices are potentially needed. Despite target sectioning however, they report that imaging experiments still lasted for about an hour. Tomanek *et al*. were able to identify most of the major internal organs of the drone, including the genitals, from the slices they acquired. They were also able to identify the ovary, crop, midgut, spermatheca, median oviduct, sting cavity, rectum and air sacs of the queen from the single sagittal cross-section. Although the images are not as visually striking as those obtainable from larger biological samples (e.g. Assheuger and Sager, 1997), it was still possible to identify a number of organs and organ systems. Furthermore, the *in vivo*, non-invasive images reveal the actual size and position of organs, which can be readily measured, in three-dimensions, within the body. Consequently, comparative studies among individuals can be performed (Tomanek *et al*., 1996). Tomanek *et al*. propose that the impact of different food regimes on glands and midgut filling and the sensitivity of different individuals (e.g. workers versus queens) to temperature, humidity or isolation could be evaluated.

Fresneau *et al*. (1991) used MRM to obtain a transverse image slice through the abdomen of the giant tropical ponerine ant, *Dinoponera quadriceps*. They obtained a single image with a slice thickness of 370 µm. They concluded that the image was encouraging: the digestive tract was plainly visible and they suggested that three-dimensional imaging gives the potential to measure the volume of anatomical structures *in vivo*. In the same study, they also present a profile image of *Drosophila melanogaster*, with a slice thickness of only 85 µm; however, the images they present are of poor quality and although "promising" do not allow for much useful biological interpretation.

A major advantage of MRI over conventional techniques in that MRI has the potential to be performed *in vivo* (discussed below). Struyf (1997) assessed the utility of *in vivo* MRM in comparison to conventional destructive microscopic techniques. *In vivo* MRM imaging of three ant species (*Pachycondyla apicalis*, *Dinoponera quadriceps* and *D. australis*) was undertaken with the same specimens being dissected, or embedded for histological sectioning, immediately after imaging. Although *P. apicalis* could be kept motionless by cooling (9-14 °C), the internal movement of organs still caused image blurring, especially within the abdomen where blurring was sufficient to prevent confident organ identification. In contrast, *in vitro* imaging of the much larger *Dinoponera* species was more successful, allowing individual structures to be distinguished in each tagma. In the head, the protocerebral and optical lobes of the brain and the mandibular gland were visible. In the thorax, the labial gland and oesophagus were poorly resolved but other structures such as the metapleural gland and ganglia of the ventral nervous system were clearly visible. Sectioning of the abdomen showed the digestive system and, in sections of a gamergate (the mated reproductive worker in these queenless ants), the ovaries were well defined.

Blurring due to internal movements is a common problem associated with *in vivo* MRM studies (discussed further below). However, Jasanoff and Sun (2002) were able to image the brain structures of unanesthetized blowflies (*135 Sarcophaga bullata*) with a voxel size of 20-40 µm without any attendant blurring. They were able to achieve this by firmly securing blowflies using wax, and by limiting their imaging to the head and thorax that contain relatively immobile tissues rather than the abdomen where gut movements can cause considerable blurring (Struyf, 1997; this study, see below). To prevent movements of the proboscis and associated musculature causing blurring in scans of the head, the proboscis was either removed or secured to the head with wax. Blowflies typically survived a day after the procedure if they were unfed and several days if fed. While Jasanoff and Sun (2002) acquire arguably the most impressive *in vivo* images yet obtained on entomological material, the procedure of securing the insects appears to have caused increased post-imaging mortality and will certainly have affected behavior. If post-imaging behavior is unimportant then *in vitro* imaging may be a preferable approach, and if normal post-scanning behavior is desired then resolution and image quality may have to be compromised for *in vivo* imaging.

Examples of MRM Images

In order to demonstrate the type of images obtainable using magnetic resonance microscopy on typical entomological specimens we imaged a dead queen of the common wasp (*Vespula vulgaris*, collected prior to nest founding in spring, 2002) and a live worker of the large ant species *Dinoponera quadriceps*. To limit movement during imaging, the *D. quadriceps* specimen was refrigerated at 5 °C for 15 minutes and then wrapped tightly in tissue paper. The specimen was then put into a 15mm (internal diameter) glass NMR sample tube that was loaded into the bore of the magnet, which was chilled to between 10.5 °C and 13 °C. Imaging data were collected at the Magnetic Resonance Centre (School of Physics and Astronomy) of the University of Nottingham on a Bruker DSX 400MHz spectrometer (http://www.bruker.com/) equipped with a super-wide bore 9.4 Tesla magnet and standard Bruker microimaging accessories. Pulse sequences were either a conventional 2D spin-

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echo image sequence or a 3D spin-echo sequence with short echo time. Parameter settings for individual images are indicated on the appropriate legend. The data were transformed and slices produced using a Silicon Graphics workstation and image processing software (IDL, RSI Systems, http://www.rsinc.com/). Three-dimensional images were produced with volume rendering as implemented in the Voltex module of Amira 2.3, a 3D visualization and reconstruction software package running on Windows 9x/ME or Windows NT/2000.

Vespula vulgaris

Images were obtained in frontal and transverse sections. Transverse sections were used to prepare three-dimensional reconstructions. Three-dimensional reconstructions and successive-slice movies are available at the hyperlink above.

The transverse sections (Fig 1A) through the head, thorax and abdomen show many internal structures clearly. In the head (Fig. 1B), the ocelli, protocerebral lobe, optic lobe, optic nerve, eye, mandibular muscles and oesophagus are all visible. The musculature of the thorax is particularly well resolved with the longitudinal and vertical indirect wing muscles prominent (Fig. 1C). Thoracic air spaces of the tracheal system are also visible. In the mid abdomen (Fig. 1D), the ovaries, and the ventriculus and hindgut of the digestive system are apparent. It is possible to discern fine details of the ventricular musculature and individual oocytes within the ovaries. As in the thorax, tracheal air sacs can be seen. In the lower abdomen (Fig. 1E), the poison gland can be seen as well as the ventriculus and hindgut.

The three-dimensional reconstructions (Figs. 3A-E) allow organs to be seen *in situ* and show spatial relationships and surface texture not apparent on the transverse slices. The gut (Figs. 3C and 3D) and thoracic musculature (Fig. 3A) are particularly striking in these reconstructions. It is possible to color parts of each section manually to provide a three-dimensional colored image of particular organs and organ systems (Fig. 3B). Structures may also be removed in this way to improve clarity and the whole image, or a part of it, may be rotated onscreen. Surface reconstructions and volume calculation can be performed by manually outlining individual organs.

Dinoponera quadriceps

Only the clearest section (a sagittal section) obtained from a live *D. quadriceps* is shown (Fig. 2). Internal structures are poorly resolved although the gut is partly discernable. This shows that imaging of live insects can still be problematic, unless one has an efficient method for preventing internal organ movements.

MRI Compared with Conventional Techniques

In vitro versus in vivo

MRI has had such a major impact on clinical diagnosis and medical research because it is possible to produce high-resolution images of internal structure *in vivo*. Because conventional entomological internal imaging techniques are only possible *in vitro,* MRI offers a real opportunity to view the internal structure of living material, and to re-sample an individual many times over an extended time period. However, *in vivo* imaging of entomological samples using MRI presents serious obstacles. While it is possible to acquire high-resolution images *in vitro* (e.g. our images of a wasp queen) attempts at *in vivo* imaging have usually produced blurred images of limited or no biological use (e.g. Struyf, 1997; our image of a *Dinoponera* worker).

The problem of acquiring useful *in vivo* images is caused by internal movements of organs and muscles. Since the image acquisition time is relatively much greater than the frequency time scale of internal movements the resulting images are blurred, rather like having an inappropriately slow shutter speed with a conventional camera. Furthermore, there is a trade-off between acquisition time and image resolution. Consequently, the problem of internal blurring gets worse as one pursues higher resolution. There are two obvious solutions to this problem. First, reduce the image acquisition time such that internal movement is effectively frozen, just as with a high shutter speed in a conventional camera. Second, reduce or eliminate internal movement.

Image acquisition time will undoubtedly be reduced as the physical technology of MRI develops, but the improvements will have to be several orders of magnitude to be of use in high-resolution entomological studies *in vivo*. Reducing or eliminating internal movement is a solution to image blurring that can be developed by entomologists with access to imaging equipment. It is essential however, that any anaesthetic technique neither damages the insect or affects post-aesthetic behavior. We used a combination of light chilling and wrapping the insect in tissue paper but this was clearly inadequate to prevent internal movement. Other researchers have anesthetized specimens with carbon dioxide (Chudek *et al*., 1998), ether (Skibbe 1995a), tetrachloroethane (Goodman *et al*., 1995) or chloroform (Goodman *et al*., 1995). Constant cooling within the bore of the magnet was achieved either at 9-12 ^{o}C (Tomanek *et al*., 1996) or at a constant 5 ^{o}C maintained by a liquid nitrogen cooling system (Fresneau *et al*., 1991, Skibbe *et al*., 1995a; 1995b, Struyf, 1997). Chloroform is a killing agent and Goodman *et al*. (1995) found that their *Pieris brassicae* larvae did not survive the treatment. They recommended a combination of chilling and a non-lethal anaesthetic like carbon dioxide for immobilization. However, carbon dioxide may have hormonal effects that affect the reproductive physiology of worker and queen honey bees (Harris *et al.*, 1996), so may not be suitable if the aim is to follow reproductive changes. We suggest that a combination of techniques (e.g. chilling combined with ether) may prove to be the most successful. Additionally, accurate targeting by pilot sectioning (Tomanek *et* al., 1996) would reduce the overall time required for image acquisition and thereby reduce the effects of anesthetics on the sampled individual. Finally, contrast agents, such as gadolinium III complexes or soluble iron compounds, are used to enhance contrast between tissues (Merbach and Tóth 2001) and could be useful here. The use of contrast agents and their long-term effect on insects have not, however, been investigated.

MRI versus dissection and light microscopy

Dissection provides a relatively rapid and cost-effective way to investigate the internal anatomy of insects. However, dissection can only be performed *in vitro*. Consequently, a single individual cannot be sampled several times, resulting in confounding inter-individual variation. Second, dissection inevitably distorts the

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internal organs and their spatial inter-relationships. Ignoring the current problems of *in vivo* MRI, *in vitro* MRI has several advantages over conventional dissection. The complete, high-resolution scan of a wasp queen abdomen took 13 hours (this study) but if time is not critical, MRI is capable of producing biologically useful two-dimensional images and three-dimensional reconstructions showing real inter-relationships between organs and muscles. If the sample is fresh, these inter-relationships are likely to reflect closely the *in vivo* situation. With suitable software, three-dimensional reconstructions can be "virtually" dissected along any plane allowing multiple dissections and unusually difficult dissection to be performed on a single sample. In addition, the volume, surface area and number of structures can be accurately determined. Images can also be enhanced and manipulated to highlight structures of specific interest or to provide computer-compatible educational images.

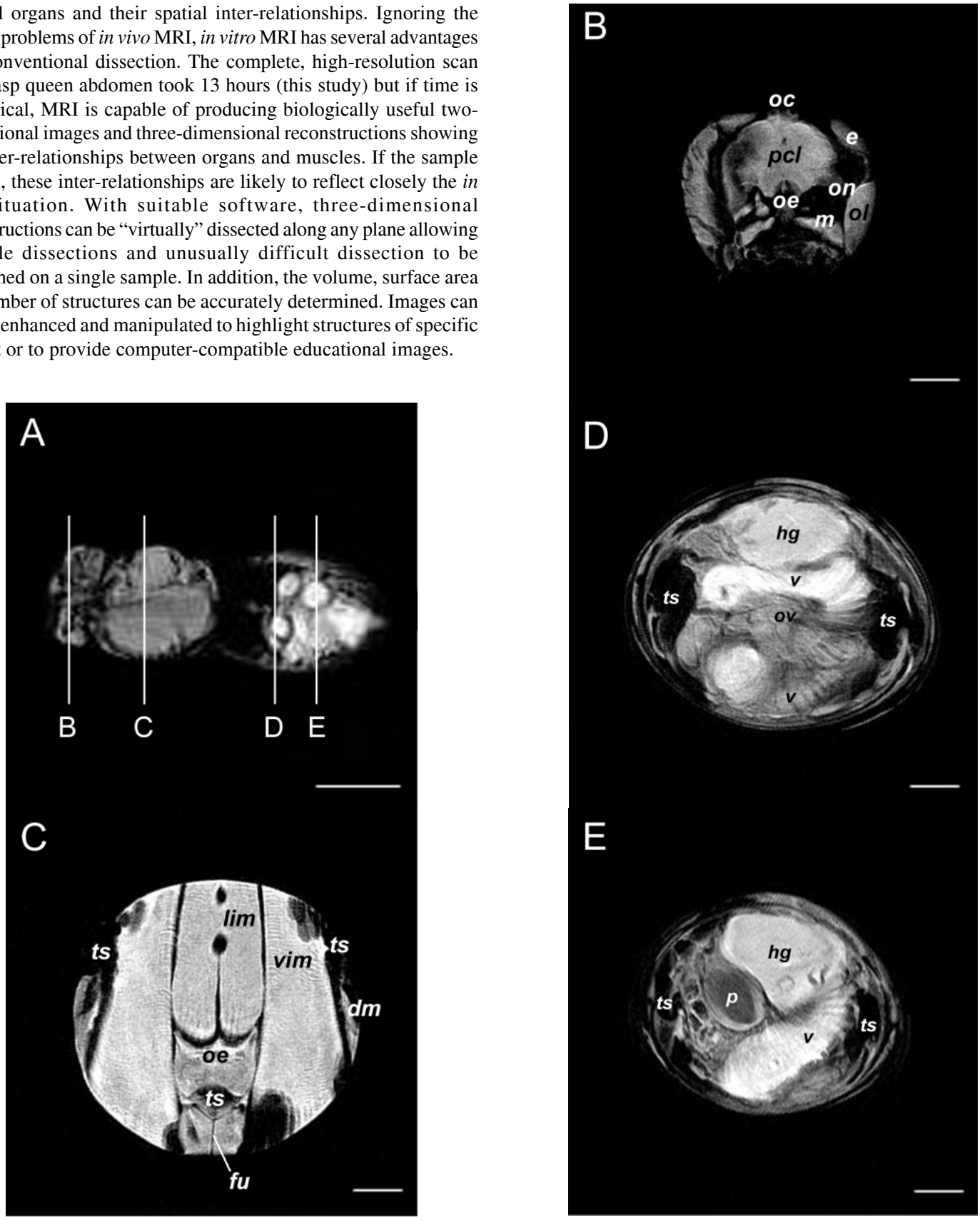
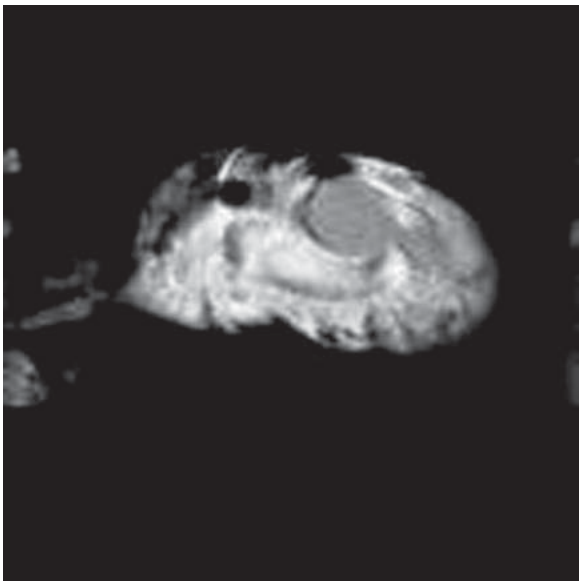


Figure 1. MRI microscopy images of a *Vespula vulgaris* queen. A is a 1000 µm thick frontal section (voxel size 101 x 101 x 1000 µm, scale bar = 5 mm, 4 averages, TR 1 s, TE 2.6 ms, acquisition time 8 minutes); B, C, D and E represent a single series of 250 µm thick transverse sections (dorsal side up, voxel size 31 x 31 x 250 µm, scale bars = 1 mm, 6 averages, TR 0.5 s, TE 2.6 ms, acquisition time per slice 13 minutes). **A** Overview with position of transverse sections indicated. **B** Transverse section through the head. **C** Transverse section through the mid-thorax. **D** Transverse section through the mid-abdomen. **E** Transverse section through the lower abdomen.

dm = direct wing muscle; *e* = eye; *fu* = furca; *hg* = hind gut; *lim* = longitudinal indirect wing muscle; *m* = muscle to mouthparts; *oc* = ocelli; *oe* = oesophagus; *ol* = optic lobe; *on* = optic nerve; *ov* = ovaries; *p* = poison gland; *pcl* = protocerebral lobe; *ts* = tracheal sacs; *v* = ventriculus; *vim* = vertical indirect wing muscle

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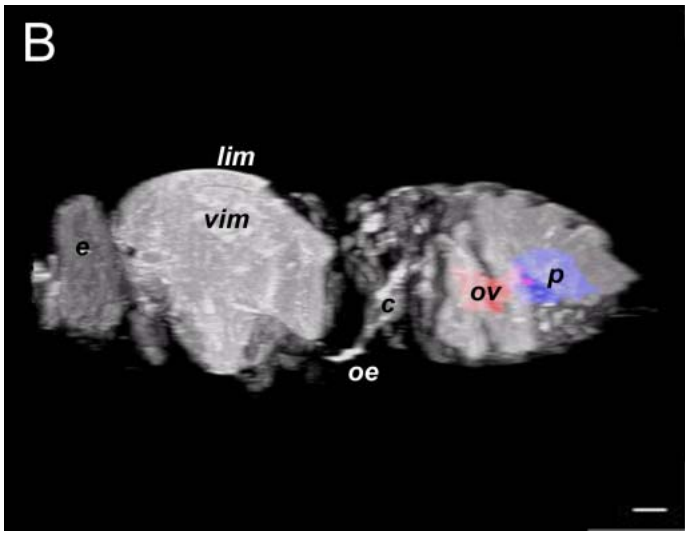
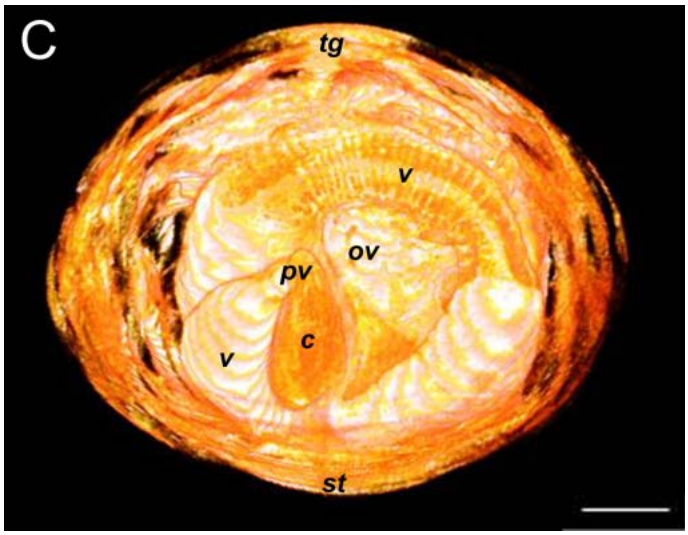
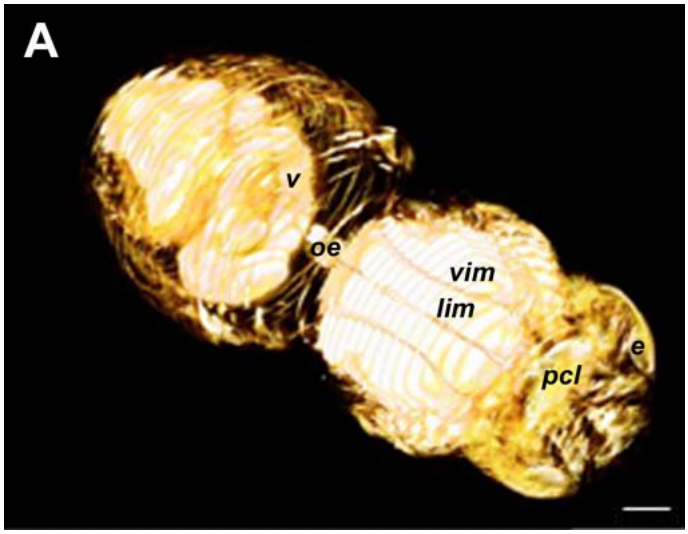


Figure 2 (Above). Sagittal section through an unmated *Dinoponera quadriceps* worker (unmated) 1000 µm thick section (voxel size 101 x 101 x 1000 µm, scale bar = 5 mm, 4 averages, TR 1 s, TE 2.6 ms, acquisition time 8 minutes. Trachael air spaces are apparent but further identification of structures is not possible with any degree of certainty.





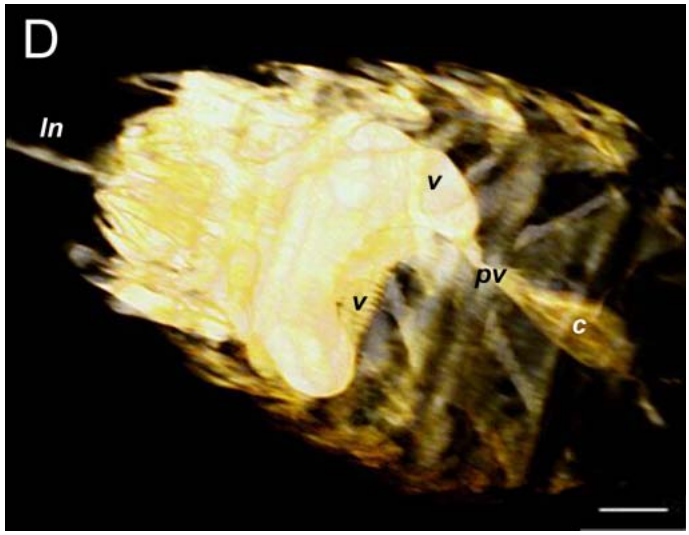


Figure 3. Three dimensional reconstructions of a *Vespula vulgaris* queen. A, B and E are based on 59 250 µm MRI slices of a whole wasp (voxel size 31 x 31 x 250 µm, 6 averages, TR 0.5 s, TE 2.6 ms, acquisition time 13 hours); C and D are based on 61 140 µm slices of a wasp abdomen (voxel size 28 x 28 x 140 µm, 6 averages, TR 0.5 s, TE 2.6 ms, acquisition time 13 hours); all are in orthogonal perspective, scale bars are 1 mm. **A (Above)** Top view. **B (Right)** Lateral view with ovaries and poison gland coloured (maximum intensity projection). **C (Right)** Anterior to posterior view of the abdomen. **D (Right)** Ventral view of the abdomen (data set interpolated from 61 to 256 slices using the Lanczos method). **E (Page 8)** Facial view of the head.

c = crop; *e* = eye; *l* = lancet; *lim* = longitudinal indirect wing muscle; *m* = muscle to mouthparts; *oc* = ocelli; *oe* = oesophagus; *ol* = optic lobe; *on* = optic nerve; *ov* = ovaries; *p* = poison gland; *pcl* = protocerebral lobe; *ph* = pharynx; *pv* = proventriculus; *sg* = suboesophagal ganglion; *st* = sternites; *tg* = tergites; *v* = ventriculus; *vim* = vertical indirect wing muscle

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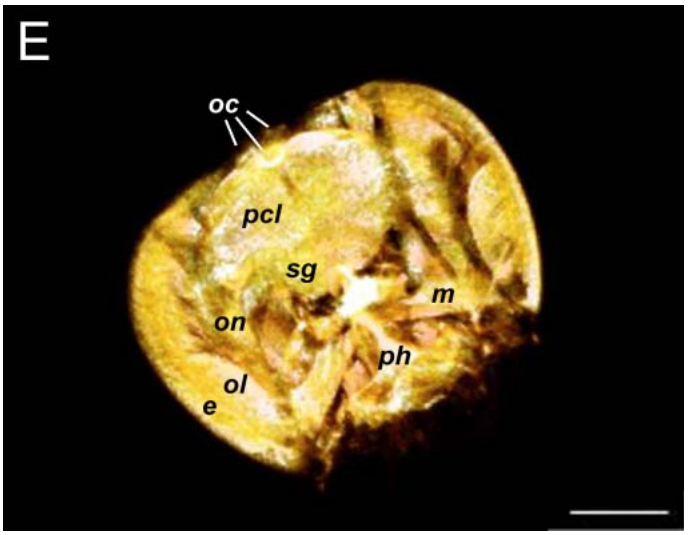


Figure 3e. (Legend on page 7)

MRI versus serial sectioning and light microscopy

MRI can produce a series of two-dimensional "slices" through a sample, which may be integrated to form a three-dimensional reconstruction. The sample is left intact after the MRI procedure. It is possible to achieve a similar result with conventional methods, using serial sectioning with a microtome to produce a series of thin slices of the sample. These slices can be scanned and then manipulated using commercially available computer hardware and software to produce a three-dimensional reconstruction. However, this procedure has a number of problems associated with it, which are absent in MRI. First, sample preparation (e.g. fixing and embedding in a hard medium) and sectioning are laborious and will result in fixation artifacts. Second, physically sectioning insects is made difficult by the hard cuticle (Tomanek *et al*., 1996). Third, sectioning is destructive and the sample cannot be re-sectioned (e.g. along a different plane). Fourth, the preparation of three-dimensional reconstructions from serial sections is both time consuming and very labor intensive, requiring sections to be digitized and aligned and structures to be outlined manually. The major advantage of conventional serial sectioning and light microscopy, however, is that it provides far higher resolution than MRI. Resolution can be increased further still by using ultra-microtome sectioning combined with electron microscopy.

Conclusions

Accepting the current shortcomings of *in vivo* imaging, MRM has been successful in providing biologically useful *in vitro* images of entomological material. *In vitro* imaging can produce both two-dimensional sections and three-dimensional reconstructions from which volumes, surface areas and other useful information can be readily extracted. Three-dimensional reconstructions are also ideal for "virtual" dissections, which could prove invaluable in research, training and education. To achieve similar results using conventional techniques would be extremely time-consuming and labor intensive. A further advantage of MRM is that specimens (both living and dead) that may be rare or difficult to acquire can be fully investigated non-destructively prior to convention investigation by dissection or serial sectioning.

In addition to visual imaging, magnetic resonance techniques can provide novel data on biological systems not available using conventional microscopic methods. Chemical shift imaging (CSI) of the ^{31}P NMR signal can, for example, provide detailed spatial information on pH within organs and organ systems. Furthermore, functional MRI, which uses MRI techniques to image the flow of blood, water and other fluids through tissues (e.g. Buxton, 2001) may eventually prove possible for *in vivo* entomological studies.

Clearly, if resolution is the critical factor then MRI and MRM are left wanting in comparison with conventional methods utilizing light (and electron) microscopy. However, MRI and MRM should not be thought of as competing with conventional techniques but rather complementing them with new ways of imaging and investigating organisms (Glover and Mansfield, 2002). Arguably, the ultimate goal of MRM, at least in entomological studies, is the rapid acquisition of high quality images *in vivo* and this goal will eventually be achieved through a combination of technological improvements in the hardware and software of MRM and methodological improvements in immobilizing living specimens. Once high quality *in vivo* images can be obtained the ability to investigate both internal morphology and function of small organisms non-invasively will ensure that MRM becomes an invaluable entomological tool.

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