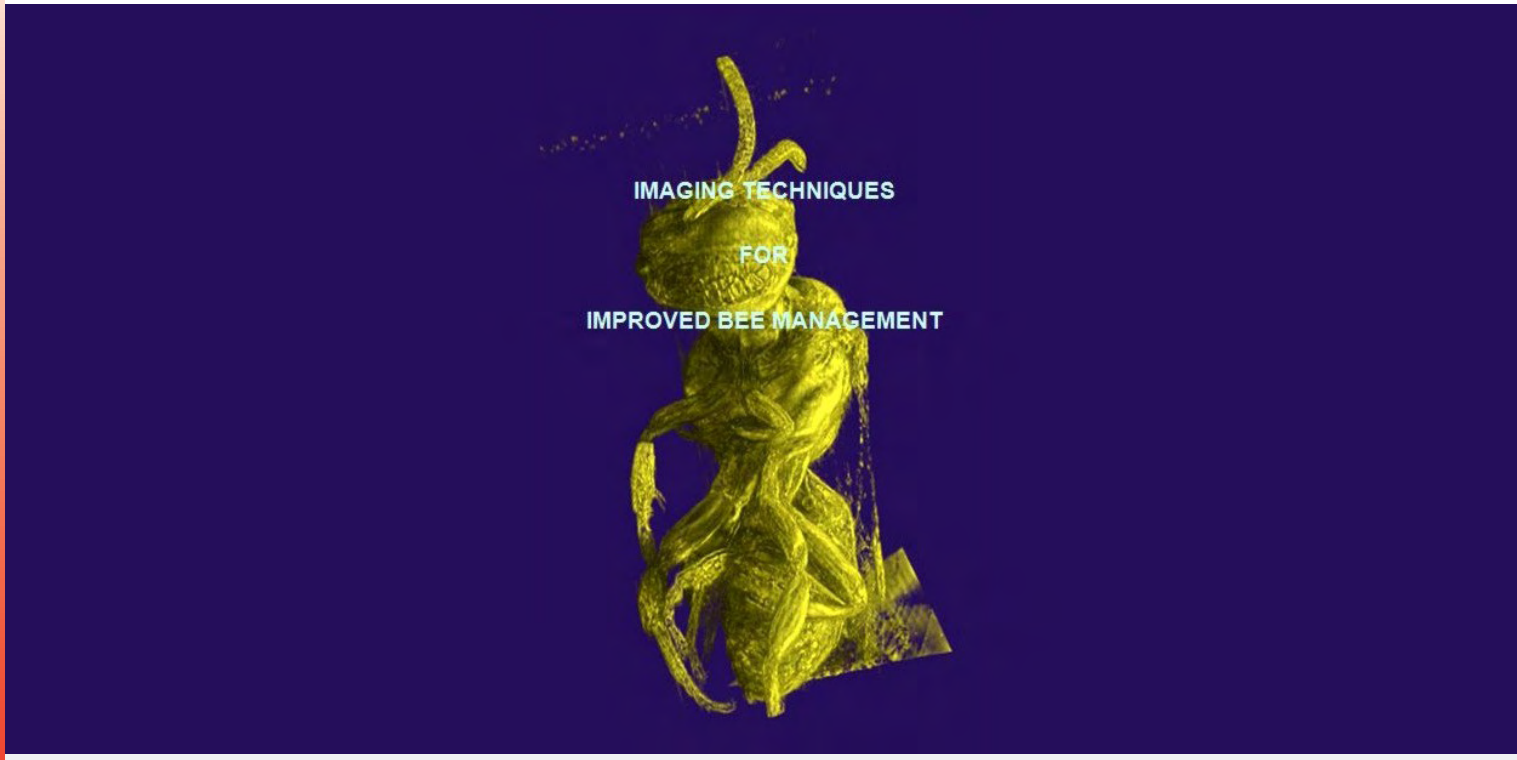
ALP science No. 534 | Juni 2010



IMAGING TECHNIQUES FOR IMPROVED BEE MANAGEMENT

Technical-scientific information

Author
Mark K. Greco
Agroscope Liebefeld-Posieux Research Station ALP
CH-3003 Berne

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Confederation

Federal Department
of Economic Affairs FDEA
Agroscope Liebefeld-Posieux
Research Station ALP

ALP is part of the ALP-Haras Unit

Agroscope

ISSN	1661-0660 (online)/ 02.08.2010
ISBN	978-3-905667-73-8
Publisher	Agroscope Liebefeld-Posieux Research Station ALP Schwarzenburgstrasse 161, CH–3003 Berne Telefon +41 (0)31 323 84 18, Fax +41 (0)31 323 82 27 info@alp.admin.ch, www.agroscope.ch
Photos	ALP; Edgar Bucher, Zurich
Layout	ALP, Müge Yildirim, 3003 Bern
Copyright	© 2010 ALP Reproduction is permitted on the condition that the source is cited and a specimen copy is sent to the publisher.

Contents

1 Introduction

Bees evolved from among the apoid wasps at least 60 million years ago (Melo, 1999; Engel, 2000; O'Toole and Raw, 2004) and extant species occur as solitary bees, semi-social bees or in eusocial colonies (Michener, 1974). The gathering of information for their appropriate classification continues, because bees are not yet formally ranked as a monophyletic group under the current classification by mellitologists (Melo and Gonçalves, 2005). Current classification places bees in the superfamily Apoidea along with wasp families such as the Sphecidae (Brothers, 1999; Melo, 1999), but with improvements in molecular analysis as a basis for their classification and as new diagnostic imaging techniques such as Scanning Electron Microscopy (SEM) arise, a definitive classification of bees as a single group is emerging (Engel, 2000; O'Toole and Raw, 2004; Grimaldi and Engel, 2005; Melo and Gonçalves, 2005). Apart from the necrophagic Trigona hypogea (Noll, 1997), the larvae of all extant species are pollen-feeding, aculeate Hymenopterans (Michener, 2000), including the Meliponinae (stingless bees). They have characteristic morphologies, such as branched or plumose body hairs, broadened hind basitarsi, many species have cleft claws. Most are solitary and approximately 5% of species are social bees living in colonies ranging from a few hundred to many thousands of individuals (Michener, 2000). Traditionally, colony health in bees has been evaluated by measuring temporal hive weight changes. However, increased hive weight can be attributed to increased pollen and nectar collection by foragers in times of colony stress (Mayack and Naug 2009). Colony health can also be assessed manually (Imdorf et al., 2001) however, due to the subjective nature of the methods, large errors can occur. Nevertheless, behavioural and morphological studies on bees continue and because many species are cryptic, have propensities to live in cavities and current methods are invasive and prone to large errors, new methods for studying them are emerging.

2 Current methods for assessing bee behaviour and colony health

The traditional methods, including the Liebefeld Method, although satisfactory for general estimations, can be misleading as well as being invasive. Increases in hive weights can indicate increases in honey stores, pollen stores, wax and forager numbers therefore, growth of colonies and good colony health. However, it has been reported that foragers in diseased/stressed colonies may exhibit precocious feeding (Mayack and Naug, 2009) and increase their nectar collection, which can actually increase the weights of hives under diseased conditions. Visual inspection can also be useful for assessing colony health, but the process of opening the hive is particularly invasive for bees. Many hundreds of bees can be killed from one observation alone. Closing the hive after visual inspection also kills bees, and places the queen at risk of being harmed, as they are squashed in the process. Therefore, it is important to develop methods which can more accurately and less invasively assess colony health.

3 Development of Diagnostic Radioentomology (DR) for assessing the behaviour and health of bee colonies

X-ray computerised tomography (CT) has been used as a non-invasive method to visualise internal and external human morphology since the early 1970s and, more recently, has been used to study soil ecology and movement in cryptic insects (Tollner, 1991; Harrison et al., 1993; Fuchs et al., 2004; Johnson et al., 2004). Modern medical MacroCT scanners can achieve image resolutions of 0.3 mm and MicroCT scanners can achieve 0.5 µm which makes CT an ideal tool for visualising internal hive components and hive structures. MacroCT scanners are useful for examining larger objects such as artificial solitary bee nests or bee hives and MicroCT scanners are useful for examining smaller objects, such as individual bees.

It has been shown in a range of pollination and bee behaviour experiments (Dainat et al., 2009; Greco et al., 2005; Greco et al., 2006; Greco et al., 2008; Greco et al., 2010b; Greco et al., 2010c; Perna et al., 2008), some of which are described later in this manual, that CT can be a useful tool for determining behaviour and health in bee colonies in managed hives (Greco et al. 2010a) and also for assessing internal and external morphology of individual bees. These results indicate that there is now the need to develop a user-friendly method for entomologists requiring greater accuracy and/or non-invasive approaches to their experiments. This new method (collectively termed Diagnostic Radioentomology - DR) is performed by utilising medical/veterinary MacroCT or MicroCT scanners (see Appendix 1). Each scanner has specific operational features that must be followed to achieve best results. Manufacturers have proprietary software that is applicable only to their scanners; however, there are a range of independent software programs available that can be used for post-processing and image analyses after the scans are performed. Thus, the information below outlines the general path which should be followed to enable DR experiments on any CT scanner currently available.

3.1 Sample preparation

One of the strengths of DR is that there is minimal or no sample preparation required. Generally, the only requirement to perform a DR experiment is that the sample needs to be positioned securely on the scanner bed for MacroCT (Fig. 1) or in the scanner sample stage for MicroCT (Fig. 2). For MacroCT of live colonies within beehives, scans generally take less 30 sec so individual bee movements do not cause any significant movement artefacts. There are also recent developments in movement correction software that will overcome these minor artefacts in the near future.

Movement artefacts are more significant when scanning live bees in MicroCT scanners. MicroCT scans of individual bees can take as long as 30 min. Even securely fastened bees continue to move slightly during scans, so it is useful to either anaesthetise them with medical grade CO2 and/or cool them down in a freezer for 10 min prior to scanning. A combination of cooling and CO2 can keep individual bees motionless for approximately 25 min. If assessment of a particular body segment is required, reducing the scan field size to that segment will take less time, thereby reducing the need for CO2 and/or cooling.

Figure 1: Sample preparation for MacroCT. Essentially the only preparation required is that the sample (bee hive) is positioned securely on the scanner bed. (Photo: V. Dietemann, ALP)

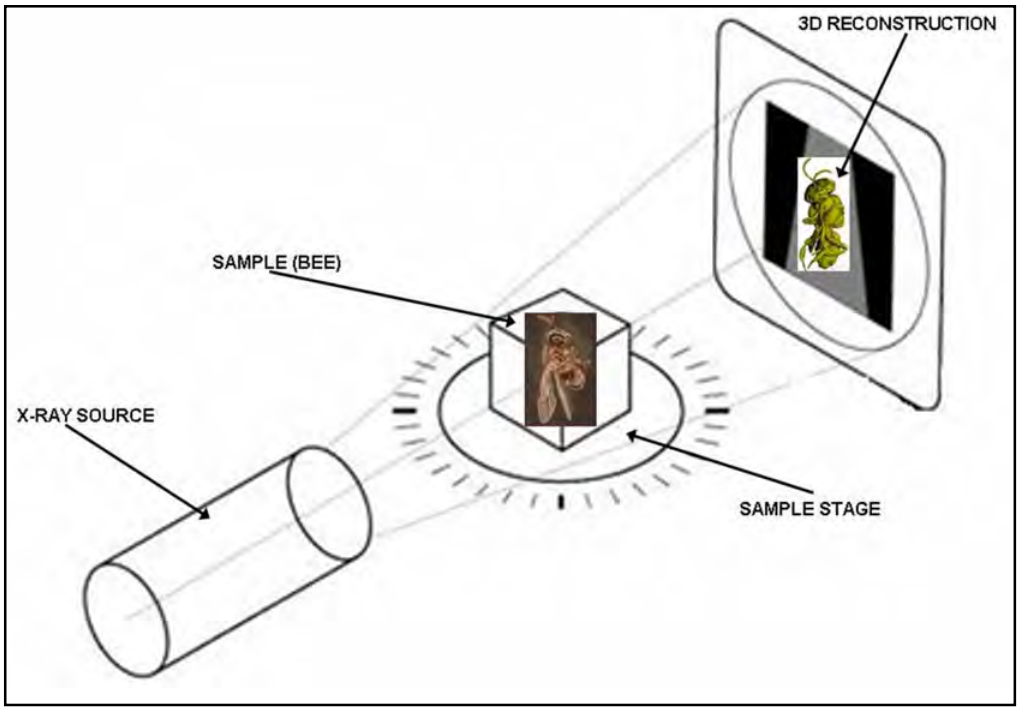


Figure 2: A schematic diagram of sample positioning for MicroCT. Essentially the only preparation required is that the sample (bee) is positioned securely on the sample stage so that the sample remains motionless during the scan.

3.2 Data Capture

Once a sample has been positioned appropriately the scanner's proprietary software will guide the user on how to perform the required scan. During the scan, the x-ray beam passes through the sample to an array of detectors that record the x-ray beam attenuations produced from the sample's unique characteristics as a digital dataset in raw volume format. The raw volume data set can later be processed to provide 2D and 3D images of the sample as well as quantitative measurements such as volume of the sample's components or structures. The initial choice of scan parameters will influence the resolution of the final 2D and 3D images and the sensitivity of the quantitative measurement's resolution. The scanner's proprietary software will offer many choices for parameter selection; however, for best image resolution and quantitative measurements, the most significant parameters to select are x-ray beam energy (amperage and voltage), scan field size, slice thickness and detector number.

3.2.1 X-ray Beam Energy

The x-ray beam's energy is determined by the amount of current (amperage) and the force of that current (voltage) selected to create the beam. The amperage determines the number of x-ray beam photons available during the scan; thus, increasing the amperage will increase the intensity of the beam. The voltage determines the x-ray beam strength during the scan; thus, increasing the voltage will increase the strength of the beam. Large A. mellifera beehives require high energy levels (120 mA at 150 kV), smaller stingless bee hives require lower energy levels (80 mA at 120 kV) and individual bees can be scanned using even lower energies (100 µA at 40 kV) to achieve appropriate image quality and quantitative measurements. For energy efficiency, x-ray dose minimisation and radiation safety it is useful to use the lowest energy levels for the required results. Generally larger and denser samples require higher energy levels than smaller, less dense samples. Entomologists will need to assess the energy level requirements independently and reduce amperage and voltage accordingly for their particular study.

3.2.2 Scan Field Size

The maximum scan field is the fixed circular area of the scanner dimensions multiplied by the fixed length of the scanner's longitudinal travel. Entomologists can select a smaller scan field size to include only the sample's region of interest. Thus, the adjusted scan field size becomes the raw volume data set in which the sample is contained. It is this raw volume data set that can be processed later to provide the 2D and 3D images and quantitative measurements. Smaller scan field areas produce better spatial resolution than larger scan field areas, because each detector will have less information to record and process. Shorter scan field travel will reduce scanning times and, thus, reduce the likelihood of movement artefacts.

3.2.3 Slice Thickness

Once the scan field size has been determined, slice thickness should be selected for the required spatial resolution. Smaller slice thicknesses result in higher spatial resolution, because there are more slices per unit of sample material. Slice thickness can be reduced to 0.3 mm for MacroCT and 0.5 µm for MicroCT. Entomologists should be aware that smaller slice thicknesses produce more slices per unit of sample material and, thus, larger datasets. Larger datasets create the need for more powerful computer hardware and software during post-processing analyses.

3.2.4 Detector Number

Higher numbers of detectors produce higher spatial resolution because there are more detectors receiving and processing information per unit of sample material. Some scanners offer a selection for the number of detectors used during the scanning process. Entomologist should be aware that higher numbers of detectors require longer scanning times. Also, more pixels per slice and, thus, larger file sizes per slice are produced. Larger file sizes create larger data sets and the need for more powerful computer hardware and software during post-processing analyses.

Once the sample has been scanned with the appropriate parameters, the resulting dataset needs to be stored appropriately. Scanners have limited space for data storage and usually auto-delete data sets that have not been stored permanently or exported. There are numerous storage devices available that can store electronic datasets (CDs, DVDs, flash drives etc.); however, external hard drives are the largest and most robust forms of data storage devices. Appropriate storage of data sets is an important component of DR which should be considered when planning an experiment. In particular, some behaviour experiments can continue for months and regular scanning can produce data sets of considerable size. A typical beehive behaviour experiment over six months can accumulate two 900 Mb datasets per month, totalling 10.8 Gb of storage space (personal observations).

5 Post-Processing

Once datasets are appropriately stored, they can be post-processed to enable sample analyses. Post-processing can be performed directly on the CT scanner; however, this prevents the use of the scanner for other examinations or another user from accessing the scanner. Therefore, remote post-processing with independent software enables entomologists to use DR ex situ. There are several post-processing software packages available which can convert CT datasets to 2D and 3D images and several that can also provide quantitative measurements (Fig. 3).

From my experience, VG STUDIO MAX V1.2 voxel data analysis and visualization software (Volume Graphics GmbH, Wieblinger Weg 92a 69123 Heidelberg Germany) and BeeView 3D rendering software (Disect Systems Ltd Suffolk, UK) produce the highest quality results and are the most versatile. BeeView is the most affordable and, for the purpose of my experiments, is the preferred choice of software. I suggest that entomologists trial the various software packages available and determine which package is the most appropriate for their requirements. Post-processing is performed as a precursor to data analysis. It is essential that post-processing is performed with both the aims and results of the experiment in mind. For example, production of quantitative volume measurements is not necessary if the experiment requires sophisticated 3D images (see section 7.0, pages: 27 & 28).

6 Data Analyses

Data analyses are dependant on the aims of the experiments and can range from simple 2D visualisation of structures to complex temporal studies of behavioural changes in colony structure or the external and internal morphology of individual bees.

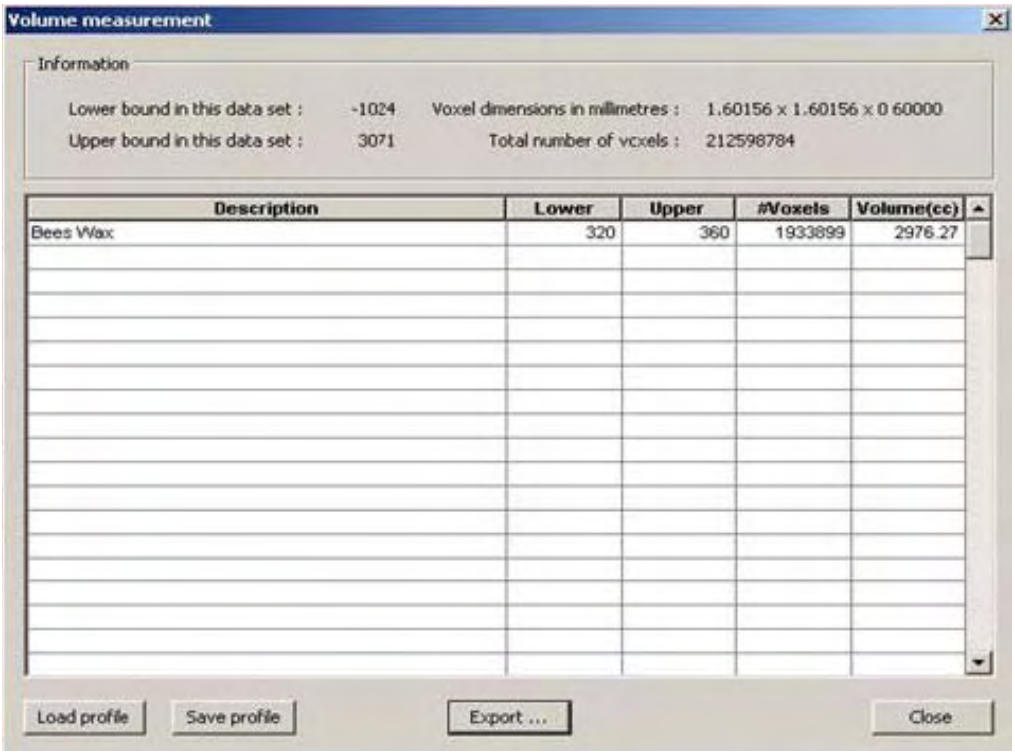


Figure 3: A BeeView post-processing table representing quantitative measurements of wax volume in a managed A. mellifera hive.

7 The use of Diagnostic Radioentomology for non-invasive observations of in-hive structures of the Australian stingless bee, T. *carbonaria*

7.1 Introduction

The involucrum in T. *carbonaria*, a reticular structure which protects the brood, prevents detailed observations of internal nest structures and evidence of queen activity. In addition, nest structures and bees are physically damaged when hives are split for observations and queen activity can only be assessed on the exposed surfaces along the cleavage site. Some Trigona beekeepers place a transparent lid under the wooden lid of the hive so they can view the colony without splitting the hive, but the bees quickly smear this with cerumen and batumen to keep light out (Dollin et al., 2001), which prevents adequate visualisation of the nest structures and queen activity.

For this experiment we used DR for non-invasive observations of T. *carbonaria* nest structures and built two- and three-dimensional scans to view and measure the involucrum, cerumen coverage and batumen within a manufactured wooden hive box (Fig. 4) containing a colony of T. *carbonaria*.

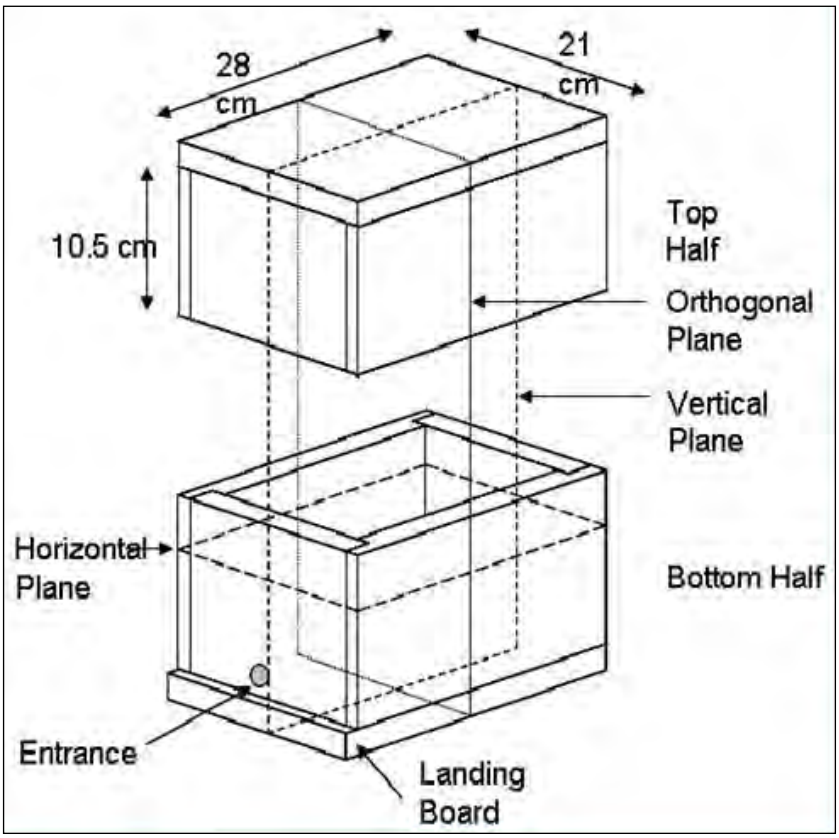


Figure 4: Diagram of the hive box designed by Dr Tim Heard for T. (reprinted with permission from the Australian Native Bee Research Centre) and showing planes of scanning.

7.2 Materials and methods

The hive entrance was sealed to prevent the bees from exiting during transport and scanning. A protective sheet was placed on the scanner (General Electric HiSpeed, General Electric Company) bed and the hive was placed on this sheet with the entrance towards the foot of the bed (Fig. 5).

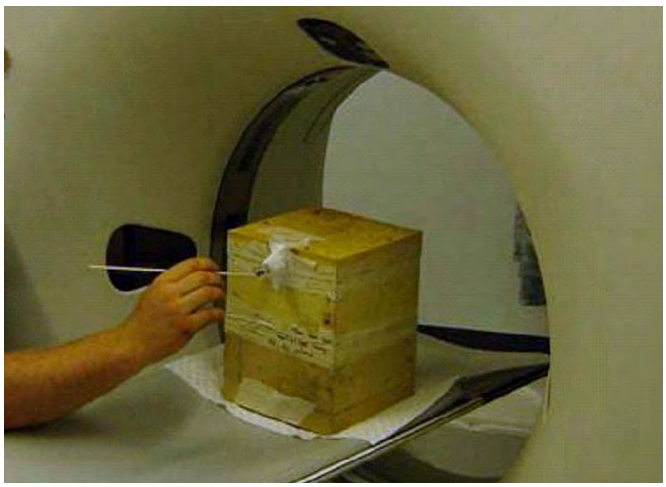


Figure 5: Sealing and positioning the hive in preparation for scanning.

The scan parameters were based on those for human paranasal sinuses (Table 1) and then modified for higher resolution imaging (Table 1); all scans were performed using these latter parameters and the hive was imaged in the vertical, horizontal and orthogonal planes. The three-dimensional images obtained were enhanced by digital subtraction of densities corresponding to wood and metal. The internal hive structures were accurately localised for scanning by utilising a lateral scout view. The brood was measured in vertical, horizontal and orthogonal planes using on-screen linear callipers. The volume of the brood chamber was then estimated using the formula 4/3r1.r2.r3 where r1, r2 and r3 are the radii of the chamber in the three planes scanned and assuming that the brood chamber was approximately ellipsoid. Further evaluation of the hive structures was performed using virtual imaging software (eFilmLite, version 1.5.0.0-DICOM, Digital Imaging and Communications in Medicine NEMA) in cine mode. Separate scans of pollen, honey and batumen were also performed to determine their level of x-ray attenuation (CT number) for direct comparison to the honey, pollen and batumen visualised within the hive.

7.3 Results

Two dimensional scans of the hive were made and nest structures in the vertical plane are shown in Fig. 6 and in the orthogonal plane in Fig. 7. These scans enabled accurate localisation of all previously reported internal nest structures (Amano et al., 2000) and, under the high resolution settings, the hive received a radiation dose of 13.9 mGy.

Honey and pollen pots were stored mainly near the entrance of the hive. The contents of these pots could be identified by their CT numbers, which corresponded to the CT numbers for the samples of honey and pollen which were +319 and -376 respectively (Table 1). Cerumen was evident throughout the hive and towards the rear of the involucrum region. The involucrum, also made from cerumen, surrounded the brood and tapered from front-top to rear-bottom of the hive. A batumen (CT number = 60) layer covered most of the inner surface of the hive and was thickest at the joins and cracks within the wood structure.

Table 1: Parameters used for imaging the T. *carbonaria* hive and its contents.

Parameter	Human paranasal sinuses	High resolution
Scout views	Two @ 90°	Two @ 90°
Slice thickness	1.0 mm	1.0 mm
Slice interval	0.5 mm	0.5 mm
Pitch	1.0 mm	0.5 mm
Peak X ray voltage	120 kV	120 kV
X ray tube current	60 mA	80 mA
Scan field of view	Small	head
Display field of view	150 mm	220 mm
Window width	+3000	+1200
Window level	+100	-695
Total scan dosage	13.9 mGy	13.9 mGy
Pixel matrix	512 x 512	512 x 512
Voxel size	0.449 mm3	0.449 mm3

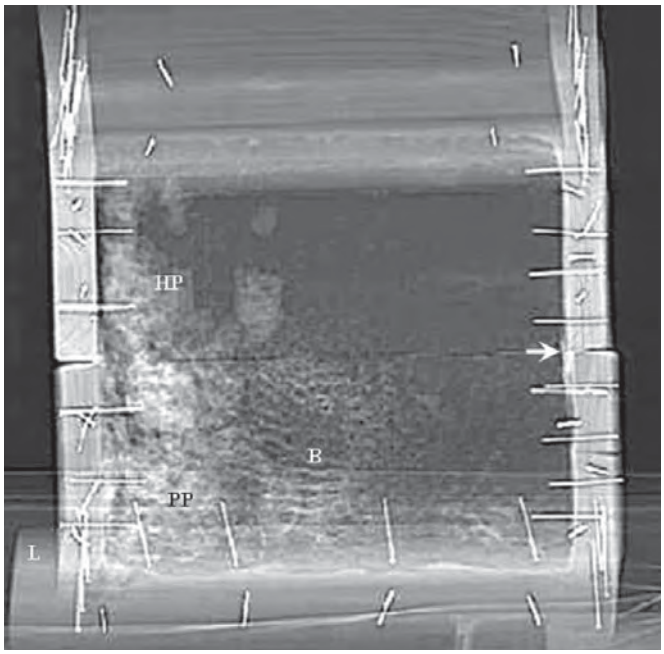


Figure 6: Two dimensional image of the hive taken in the vertical plane showing the landing board (L), honey (HP) and pollen (PP) pots, the brood chamber (B) and batumen (arrowed).

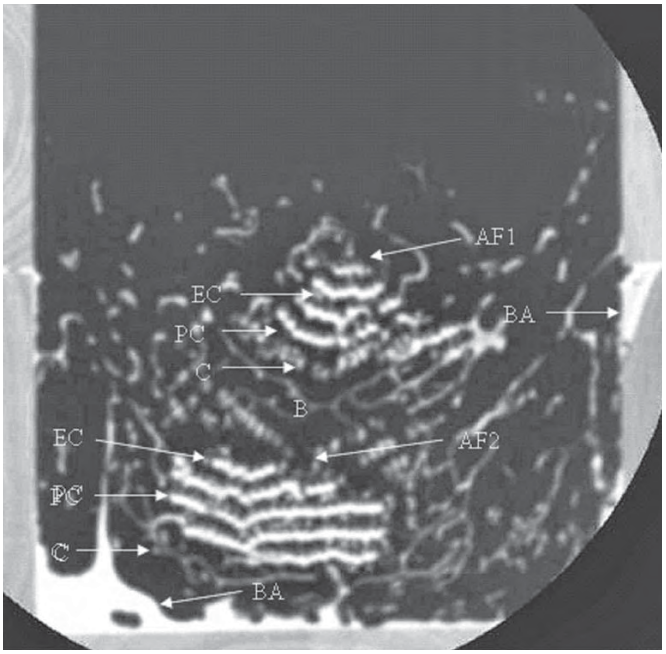


Figure 7: Two dimensional image taken in the orthogonal plane of the brood chamber (B) showing egg cells (EC) in two advancing fronts (AF1 and AF2), pupal cells (PC), batumen (BA) and final stage cocoons (C).

The 2D images allowed the size of the brood chamber to be assessed. The chamber was approximately ellipsoid in shape measuring 158 mm in height, 116 mm in width and 122 mm in length and was estimated to be 117 mL in volume. Within the brood chamber, egg cells, pupal cells and cocoons were separately identifiable (Fig.7).

In this hive, the brood consisted of two spiral shaped structures (Fig. 7 and 8) in which individuals within the brood progressed through developmental stages; cocoons were visualised at the bases, followed by pupae, through to advancing fronts of newly laid eggs. The lower and upper advancing fronts of eggs were 29 mm and 83 mm, respectively, from the bottom of the hive box.

Computer-generated, three dimensional images are shown in Figs 9 and 10. These images give a clear representation of the positional relationships between the hive structures. In Fig. 10, the spherical nature of the pollen and honey pots is clearly evident as is the grouping of most pollen pots together close to the entrance of the hive and the grouping of the honey pots above them and further to the centre of the hive. Also evident is the ellipsoid shape of the brood chamber and the surrounding involucrum. Within the brood chamber is a region between the lower layers of egg cells and the upper layers of cocoons that appears less dense. Fig. 9 also clearly shows the relationship between the brood chamber and the other structures of the nest and which in this case is approximately one-third of the occupied region of the hive box.

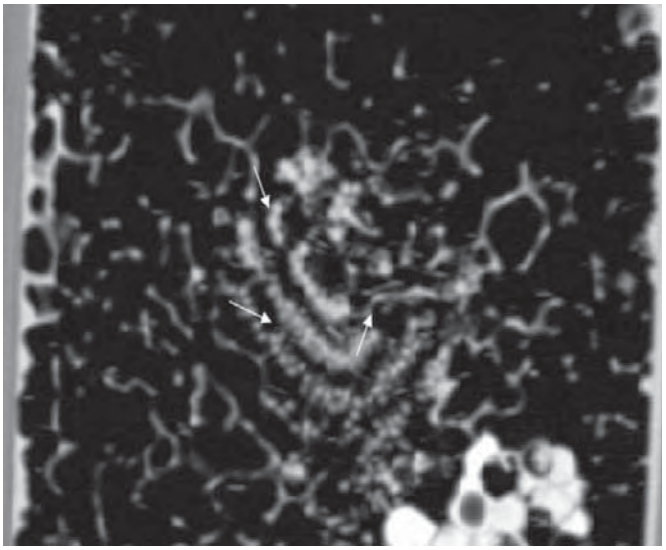


Figure 8: Two dimensional image of the brood showing spiral structure (arrowed), a feature that is characteristic of T. *carbonaria*.

Figure 10 represents an image of the hive as if viewed looking into the brood chamber through the base of the hive box. In this image a bridge of batumen is evident rising from the base and side wall of the box. The bridge was 52 mm long, curved in shape and extended in to the centre of the brood chamber.

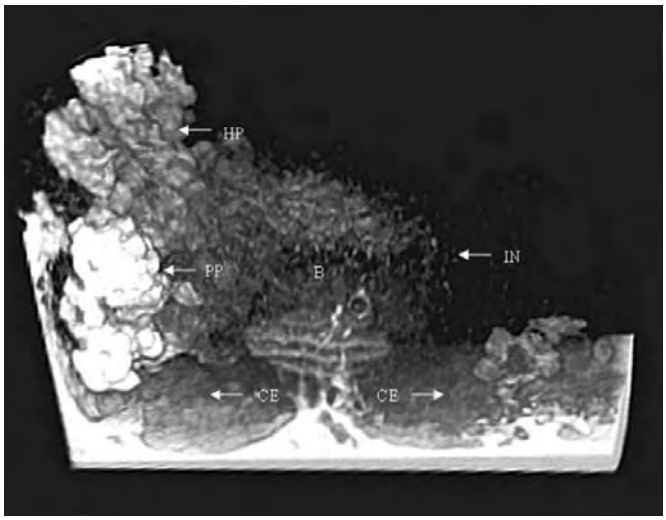


Figure 9: Three dimensional reconstructed image detailing honey (HP) and pollen (PP) pots, brood chamber (B), cerumen (CE) and involucrum (IN).

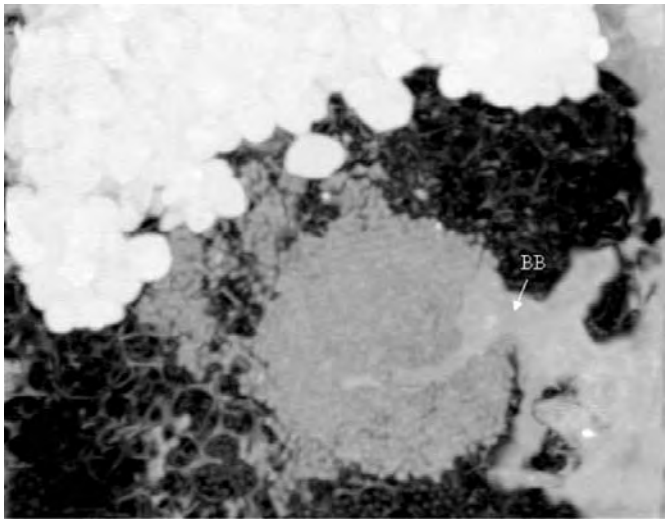


Figure 10: Three dimensional view of the batumen bridge (BR) extending into the brood chamber.

7.4 Discussion

The work presented here has been published in 2005 in the Journal for Apicultural Research which is a peer reviewed internationally recognised journal. DR allowed the internal structures of the hive to be viewed and assessed non-invasively. This new approach is non-destructive to the hive, does not modify the bees' behaviour and does not kill them which is in contrast to the traditional method of splitting the hive apart to view internal structures. In particular, because of the ability to accurately calculate brood chamber volume and structural components such as egg cells, pupal cells and cocoons, it is possible to assess queen activity and, therefore, colony health. Also, through a series of temporally spaced scans, it would be possible to monitor changes in brood chamber volume over time to assess colony health more accurately than the current methods using hive weight.

The total dosage of radiation for the scan was 13.9 mGy which was spread over 477 1-mm thick tomographic slices. Assuming that the bees remained still and were positioned perpendicular to the X-ray beam, the maximum exposure of an individual bee would be 0.14 mGy. This dosage is ~3800 times less than the minimal dose required for a biological effect to occur in Drosophila melanogaster (Kanao et al., 2003). D. melanogaster are about half the size of T. *carbonaria*; therefore, the radiation dose absorbed by each unit mass of the bee is less, and this reduces the risk of a biological effect on scanned bees even further.

The batumen bridge found in this study has not been reported before. It may be an anomaly for this particular hive but could also be a normal structure in T. *carbonaria* hives that, as yet, has not been identified and may secure the brood chamber to the base of the hive box. Further scans of a series of hives will enable us to determine whether this structure is common and, if so, what role it may have in nest architecture.

As the developmental stages of individuals within a colony can be identified, DR could be used to follow the lifecycle of stingless bees from egg to imago and may be useful for determining interactions between development and environmental factors (Dollin, A. 2004 pers. comm.). Whilst DR was used on a manufactured hive box in this study, in the future it could be used for scanning natural nests. Many stingless bee nests are found in dead logs (Michener, 1961; Dollin et al., 1997) and DR would provide a non-invasive technique to identify internal nest structures and colony behaviour. Members of Trigona are morphologically similar to each other, and it is difficult to distinguish one species from another (Green et al., 2001). However, Trigona species have characteristic nest structures (Dollin et al., 1997). CT showed the spiral nature of the brood which is characteristic of T. *carbonaria*; therefore, DR would be particularly helpful in non-invasively distinguishing this species from other species of Trigona. This ability to distinguish between species can be used to aid our understanding of their behaviour and ecology and may help conservation management programs.

8 Non-invasive monitoring of Amegilla holmesi nest structures, nesting behaviour, and adult female activity

8.1 Introduction

Bluebanded bees, Amegilla (Zonamegilla) spp. (Hymenoptera: Apidae), are medium-large, pubescent, long-tongued bees that are found mostly in coastal regions of all states of Australia except the island of Tasmania. They are solitary, fossorial bees that nest gregariously in vertical burrows in the ground or horizontally in soil embankments and occasionally in sandstone or artificial substrates (Rayment, 1947; Michener, 1961; CSIRO, 1991). Nesting sites often persist for many years and may contain thousands of cells due to the bees' natural tendency to return to their natal sites (Michener, 1960; Cardale, 1968). Studies of bluebanded bees have shown that they are active during the warmer months in Australia from early spring until the end of summer (Rayment 1947) and overwinter as prepupae (Rayment, 1947). Prepupae are larvae that have consumed all their provisions and defecated, but have not yet pupated and do not have distinct cranial, thoracic, or abdominal regions (Rayment, 1947). Investigations of the in-nest biology of bluebanded bees have been limited due to their fossorial nesting behaviour. However, with recent research demonstrating their potential as pollinators of greenhouse tomatoes (Bell et al., 2006), the ability to evaluate artificial nests is of considerable importance for the development and management of crop pollination regimes using bluebanded bees.

For this experiment, we aimed to use DR to view and measure natal cells, cell provisioning, and tunnels in nests of A. holmesi Rayment within artificial mud brick nests and to produce non-destructive, non-invasive methods to view the internal contents of nests to determine the relational organisation of individual nests and to monitor such things as the health and aggregation strengths of fossorial species.

8.2 Material and methods

Thirteen artificial mud-brick nests were constructed from PVC down-pipe and filled with mud that was made by mixing soil taken from a natural nesting site with water. The mud was dried to create the soil substrate in which fossorial bees could build their nest structures. To encourage bee nesting behaviour in the bricks, two 8-mm holes were made by pushing a piece of dowel into the front surface of the mud to an approximate depth of 40 mm. The nests were then placed at an active bluebanded bee nesting site in Hazelbrook, New South Wales (30° 13' S, 152° 43' E) in August 2004, where they remained for most of the bees' active season (early September until the end of February). Two weeks after cell provisioning was evident by adult, female bees (indicating that the nests were active and likely to contain a range of developmental stages), the nests were removed from the outdoor nesting site and placed in cages for approximately 1 h to allow any bees within the nest to emerge. The emergent bees were returned to the nesting area and the artificial nests were placed in cardboard boxes to prevent any remaining bees from escaping during transport to the x-ray facility or during scanning. A protective sheet was placed on the human body scanner bed (General Electric HiSpeed, General Electric Company 3135 Easton Turnpike Fairfield, CT 06828-0001 United States). The scanner was a single slice total human body scanner used for imaging bone and soft tissues in humans. The artificial nests were positioned on the sheet with the front end towards the foot of the bed: the nests were scanned on 4 February 2005. Each nest was scanned separately and the scanner instrumentation indicated each nest received a radiation dose of 7.7 mGy. The scan parameters used for high resolution imaging (Table 2) were based on those for a human wrist. Wrist parameters were chosen, as it was estimated that the dimensions and x-ray attenuation characteristics of a human wrist are similar to the dimensions and attenuation characteristics of the soil in the artificial nests.

Table 2: Standard human wrist protocol and modified high resolution protocol for A. holmesi artificial nest scanning

Parameter	Human wrist protocol	High resolution protocol
Scout views	Two @ 90°	two @ 90°
Slice thickness	1 mm	1 mm
Slice interval	0.5 mm	0.5 mm
Pitch	1.0 mm	0.5 mm
Peak X ray voltage	100 kV	120 kV
X ray tube current	50 mA	60 mA
Scan field of view	small	large
Display field of view	150 mm	220 mm
Window width	+3000	+1499
Window level	+100	+269
Total scan dosage	7.0 mGy	7.7 mGy
Pixel matrix	512 x 512	512 x 512
Voxel size	0.449 mm3	0.449 mm3

The internal nest structures were accurately located for scanning by utilizing two scout views. The scout views allow the operator to plan a scan sequence according to the structures visualised in these views and enables the operator to use a smaller scan field which increases resolution and minimises the radiation dosage by not scanning extraneous regions. Evaluation of internal nest structures was performed using virtual imaging software in cine mode (eFilmLite version 1.5.0.0-DICOM, Digital Imaging and Communications in Medicine NEMA, suite 1752 1300 North 17th Street Rosslyn, VA 22209). The natal cells and tunnels were measured in horizontal, vertical, and orthogonal planes using on-screen linear callipers: means with their standard deviations are presented and were determined using SPSS (V11.5). The CT image files produced were then used to reconstruct 3D images of the nests and their internal structures. Three algorithms, scatter HQ, sum-along-ray, and false colour (VG STUDIO MAX V1.2 voxel data analysis and visualization software, Volume Graphics GmbH, Wieblinger Weg 92a 69123 Heidelberg Germany) were compared to evaluate their benefits for optimum visualization. After scanning, one nest was physically split to confirm the DR interpretations. The x-ray attenuation values (Hounsfield units, Hu) corresponding to different nest structures and contents were determined and the three-dimensional images were further enhanced by digital subtraction of all regions with x-ray attenuations corresponding to the soil substrate (+201 to +900 Hu).

8.3 Results

Two-dimensional scans of the structures of a typical nest in the horizontal, vertical, and orthogonal planes are shown in Fig. 11. These scans enabled accurate visualization of all previously reported nest structures (Cardale, 1968; Michener, 1960; CSIRO, 1991) including natal cells with and without provisions, tunnels, larvae, pupae, parasitic fly larvae, and colonies of an invasive fungus. The contents of natal cells could be identified by their differing physical characteristics. Within these cells, larvae could be distinguished from pupae as the former have a distinct crescent shape whilst the latter have a well-defined head, thorax, and abdomen (Fig. 11A).

A distinct, characteristic interface with a concave meniscus, not previously reported, was seen between the liquid provisions and the air within those cells that appeared to contain only liquid provisions (Fig. 11c). When the nest was physically examined, eggs were found in the three cells that had a meniscus evident in the scans. In cells that contained larvae, pupae, or the fungus, Ascosphaera sp., in addition to liquid provisions, the concave nature of the interface was not observed. Figure 11A shows a cell containing puparia of the cleptoparasitic fly, Miltogramma sp., which were present in a few cells from all nests. Cells containing Miltogramma sp. could be distinguished from those containing A. holmesi as, firstly, cells containing the former species always had 2–4 insects in each cell whereas those containing the latter species only had one individual. Secondly, the puparia of the two species differ in shape: the puparia of Miltogramma spec. are maggot-shaped whereas those of A. holmesi are crescent-shaped. Thirdly, the puparia of Miltogramma sp. were 7 ± 0.84 mm in length and 2 ± 0.07 mm in diameter (n = 10) and were smaller than those of A. holmesi (9 ± 0.85mm in length; 4 ± 0.09mm in diameter; n = 10). Lastly, the puparia differ in x-ray attenuation; for Miltogramma spec. +10 to +150 Hu and for A. holmesi spec. +44 to +120 Hu (Fig. 4.12). Also, all nests had some cells containing the cell-invading fungus, Ascosphaera sp. (Fig. 4.11B). After scanning, 12 of the nests were returned to their original site. All were re-accepted by the bees and natal cell provisioning recommenced.

Larvae could also be distinguished from pupae due to differing x-ray attenuation (Fig. 12) and by the absence of provisions within pupal cells.

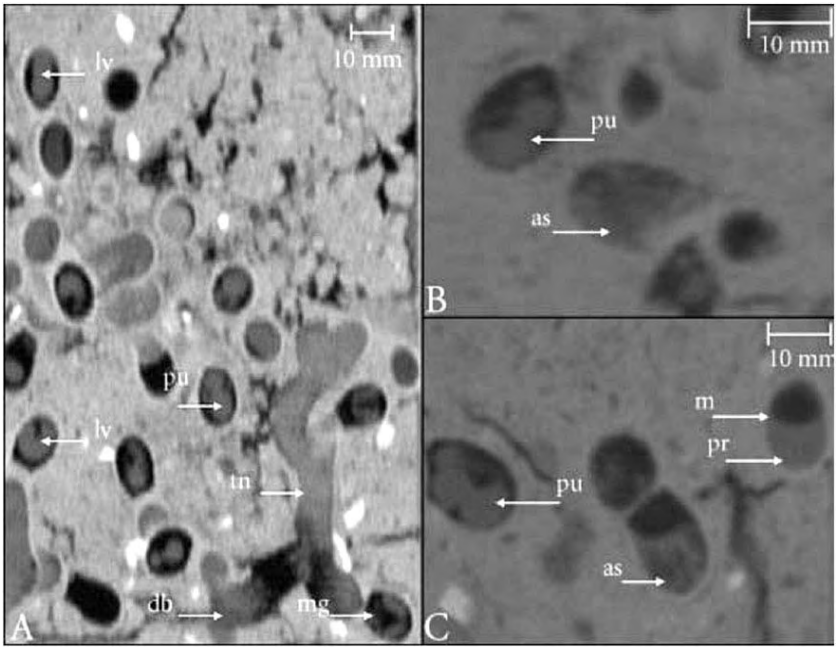


Figure 11: Two-dimensional CT images of an artificial nest of Amegilla holmesi in the (A) horizontal, (B) longitudinal, and (C) orthogonal planes showing cross-sections of natal cells (nc) containing provisions (pr) including the concave nature of the surface meniscus (m) of the liquid provisions, tunnels (tn) including debris (db), Amegilla larvae (lv), Miltogramma spec. (mg), Amegilla pupae (pu), and colonies of an invasive fungus, Ascosphaera sp. (as).

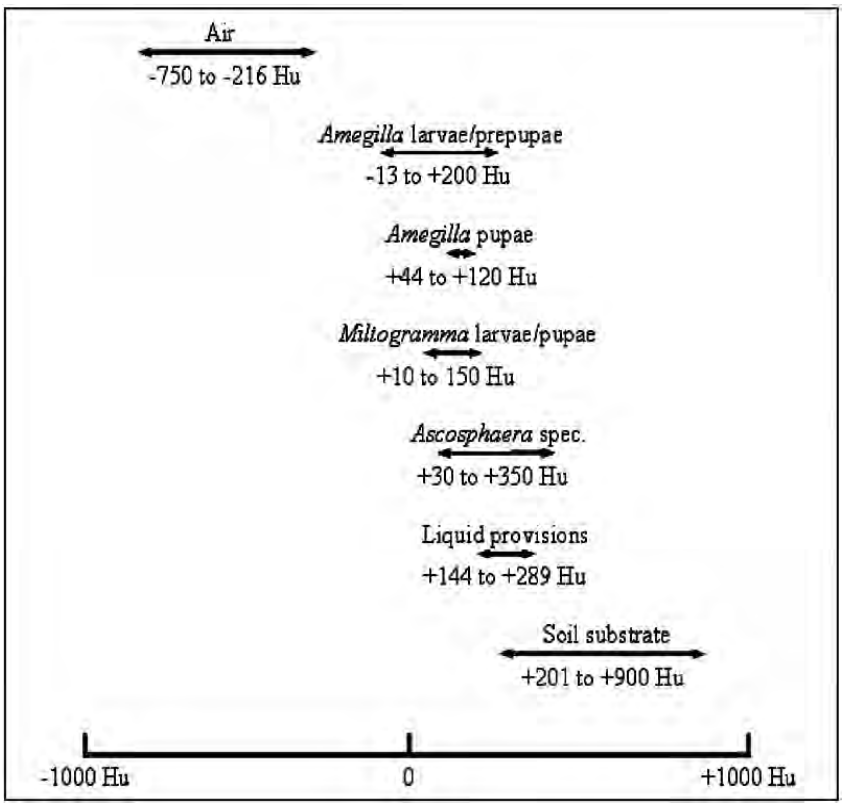


Figure 12: X-ray attenuation values for artificial nests contents of Amegilla holmesi in Hounsfield Units (Hu).

Individual nests were found to contain between 40 and 60 larvae (n = 715; mean 55 ± 6.15) and pupae and between eight and 28 (n = 247; mean 19 ± 7.39) provisioned cells. Prepupae were most abundant reflecting the time of year when the nests were assessed, that is, towards the end of the bees' active season. Eggs of A. holmesi were present when the nest was split open and visually assessed; however, they could not be visualised in the DR images.

In addition to cell contents, the 2D images in all three imaging planes allowed the size of cells and tunnels to be assessed non-invasively for the first time. Five cells were randomly selected from one nest and their internal dimensions were measured. Their mean length and diameter were 10.8 ± 0.1 and 8.0 ± 1.01 mm, respectively. Also, five tunnels were randomly selected and measured for mean internal length and diameter, which were 42.6 ± 7.84 and 7 ± 0.28 mm, respectively.

Nest structures were also visualized using computer-generated, three-dimensional imaging. Three reconstruction algorithms were used to evaluate their benefits for best visualisation results: scatter HQ (Fig. 13B), sum-along-ray (Fig. 13C), and false colour (Fig. 13D). The scatter HQ algorithm allowed structures along a section of the nest to be visualized and permitted the nest to be digitally dissected to reveal the structures along any of the three planes of the image. Using scatter HQ, tunnels and natal cells can clearly be seen. The sum along ray image permitted the contents within tunnels and natal cells to be viewed; larvae and pupae could easily be recognized within cells as could debris within tunnels. After digital removal of the soil substrate segment of the image (+201 to +900 Hu), the application of false colour to individual structures with different x-ray attenuations, such as natal cells and tunnels, enabled visualization of these structures; however, contents within cells and tunnels could not be visualised.

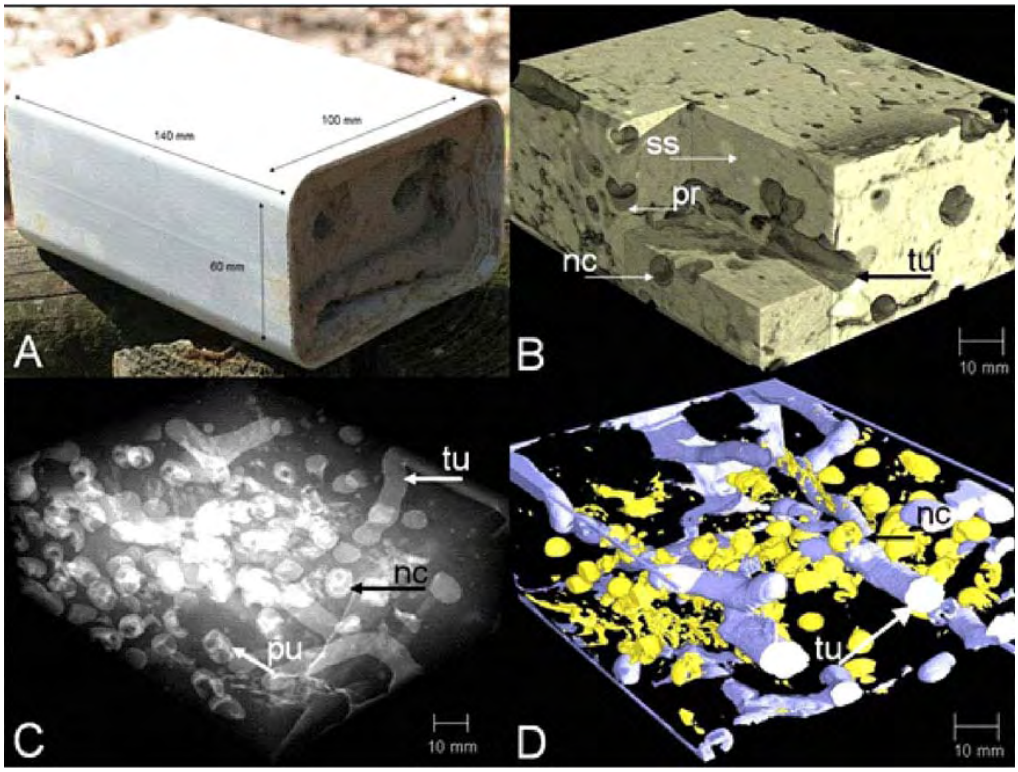


Figure 13: Three-dimensional images of the artificial nest of Amegilla holmesi used during scanning. (A) photograph of the artificial nest showing its external surfaces and their dimensions; (B) 3D scatter HQ image of the nest showing nest structures including natal cells (nc) with some containing provisions (pr), tunnels (tu), and soil substrate (ss); (C) 3D sum along ray image of the nest showing nest structures transparently, including tunnels (tu) and natal cells (nc) with some cells containing pupae (pu); and (D) 3D false colour image of the nest including digital subtraction of soil substrate which enables better visualisation of spatial relationships between nest structures such as tunnels (tu) and natal cells (nc).

8.4 Discussion

DR allowed the internal structures of the artificial nests of A. holmesi to be viewed and assessed non-invasively. The work presented here has been published in 2006 in the journal Entomologia Experimentalis et Applicata which is a peer reviewed internationally recognised journal. This new approach is not destructive to either the nest structures or the bees, in contrast to the traditional method of nest assessment that requires separating nest contents manually to view internal structure. In particular, as we used high resolution, two-dimensional imaging and improved on Tollner's (1991) use of the sum along ray algorithm by including scatter HQ and false colour, we were able to accurately localize and identify nest structures and to determine the relational organization of individual nests.

With the exception of eggs of A. holmesi, all previously reported nest contents were visualised in the DR images and the method also permitted the activities of adult bees to be assessed. Whilst eggs of A. holmesi could not be visualised in DR images, DR can demonstrate evidence of oviposition due to the visualisation of a meniscus on the top of the liquid provisions within a sealed cell. This meniscus has not previously been reported, and its presence indicates an adult bee has completed provisioning and oviposition within a cell. This cell can now be tracked non-invasively over time to observe juvenile development. Therefore, DR allows the monitoring of provisioning prior to oviposition and juvenile development post oviposition. Consequently, through a series of scans over an active season, it would be possible to follow changes in juvenile bee numbers and their growth and to accurately track changes in nest status without disruption or damage to managed nests. Artificial nests can be transported easily to research facilities that have scanners. Each scan including image processing takes less than 30 min per nest and currently costs less than AUD 20.00, which is within the reach of apiculturists and it is possible to scan several nests simultaneously, thus, lowering the costs even more.

The accurate visualisation of cell contents and cross referencing with their attenuation values enabled the incidence of parasitism by Miltogramma sp. and outbreaks of Ascosphaera sp. to be identified. Volume rendering using x-ray attenuation values corresponding to Ascosphaera sp. would enable levels of infestation to be quantified non-invasively. Therefore, nest health can be monitored using DR allowing fungal outbreaks in managed nests to be localised and infected nests or cells to be removed, destroyed in situ or treated with fungicides.

The total dosage of radiation for the scan was 7.7 mGy, which was spread over 270, 1 mm thick, tomographic slices. Assuming that larvae or pupae were positioned perpendicular to the x-ray beam (which represents the position in which a bee would receive maximum exposure during the scan) and that the average length of a bee is 9 mm, the maximum exposure of any individual would be 0.26 mGy. This dosage was estimated to be approximately 7000 times less than the minimal dose required for a biological effect to occur in Drosophila melanogaster (Kanao et al., 2003). As the mean weight for adult D. melanogaster is 1.29 mg (Katz & Young, 1975) and mean weight for adult Amegilla spp. is 1500 mg (Alcock et al., 2005), the radiation absorbed per unit mass of bees in this study is much less than for D. melanogaster or T. *carbonaria* which has a mean weight of 2 mg (personal observations). Although early developmental stages of A. holmesi might be more sensitive to radiation, the low dosage used in each scan should permit this technique to be used repeatedly in future studies without biological effects on the colony.

Nest structures were also visualized using computer-generated, three-dimensional images. These images gave better representation of the positional relationships between the internal nest structures than the 2D images. Three reconstruction algorithms were used to evaluate their benefits for best visualisation results: scatter HQ sum along ray, and false colour. The scatter HQ image gave the truest image of the nest in that both the soil and the structures within it (tunnels and natal cells) could clearly be seen. Although, the image is not transparent in that only the surface of the slice is visible and structures that lie beneath the surface cannot be visualised, scatter HQ does, however, allow continued sectioning to reveal all structures within a nest. The use of the sum along ray algorithm overcomes this lack of transparency throughout the nest. The technique also permits the contents of tunnels and natal cells to be viewed. Larvae and pupae can easily be recognized within cells as can debris within tunnels. The drawback with the images produced using this algorithm is that they do not give the best relational representation between structures within the nest. After digital removal of the soil substrate, the images produced using false colour enabled the best visual separation of the nest structures; however, contents within them could not be seen. Therefore, to obtain a comprehensive evaluation of the nest status, all 3D techniques should be used in conjunction with two-dimensional images, as each 3D technique has a different diagnostic value which permits different aspects of the nest to be visualised.

As the developmental stages of individuals within a nest can be identified, DR could be used to non-invasively follow the lifecycle of managed fossorial bee species from egg to imago. For accurate reproduction of these types of scans, the researcher should use similar window widths, window levels and scan parameters as described in this paper. DR will also be useful for non-invasively determining interactions between developmental and environmental factors by monitoring juvenile development and adult bee activity during varying environmental conditions, seasons or years (A Dollin, pers. comm.). Therefore, DR can provide a better understanding of the abiotic requirements of fossorial species and this knowledge can be used for the production of better bee management regimes and the further development of managed bee species as valuable alternative pollinators.

9 The use of Diagnostic Radioentomology to study pseudo-mummification of Aethina tumida by T. *carbonaria*

9.1 Introduction

Social insects live in colonies and construct nests which are often attractive to parasites. Some parasites feed on stored food or brood and can destroy colonies (Schmid-Hempel, 1998; Neumann et al., 2001) thus generating the need for efficient defence mechanisms. While some Coleopteran nest intruders are harmless (Lea, 1910; Lea, 1912; Neumann and Ritter, 2004; Haddad et al., 2008; Hoffmann et al., 2008), others can be damaging parasites (Neumann et al., 2001). Parasitising beetle species pose particular difficulties for their social insect hosts because their hard exoskeletons protect them from defence strategies such as biting or stinging. The small hive beetle, Aethina tumida (Coleoptera: Nitidulidae), is a parasite and scavenger of honeybee (A. mellifera) colonies endemic to sub-Saharan Africa (Lundie,1940; Neumann and Elzen, 2004; El-Niweiri et al., 2008; Neumann and Ellis, 2008). It has become an invasive species (Elzen et al., 1999; Elzen et al., 2000) with well established populations in North America and Australia (Neumann and Ellis, 2008; Spiewok et al., 2007). It lives within A. mellifera nests and feeds on brood, stored food and dead bees (Lundie, 1940; Schmolke, 1974; Neumann and Elzen, 2004; Spiewok and Neumann, 2006a). Frequently, the feeding small hive beetle larvae cause the complete destruction of the nest (Lundie, 1940; Neumann and Elzen, 2004); however, the presence of adult small hive beetles alone can be detrimental to colonies of European honeybees (Ellis et al., 2003a). This obviously creates demand for efficient defence mechanisms against intrusion and reproduction by adult small hive beetles.

Unlike other parasites, small hive beetles are easily detected and can be vigorously attacked by honeybee workers (Elzen et al., 2001). Nevertheless, because of their hard exoskeletons and defence strategies (Neumann et al., 2001; Neumann and Elzen, 2004) adult small hive beetles bypass the bees' primary defences (Lundie, 1940; Neumann and Elzen, 2004) and are subsequently difficult to be killed or ejected (Lundie, 1940; Neumann et al., 2001). Cape honeybees, A. m. capensis, display alternative defence mechanisms by encapsulating adult small hive beetles in tombs (Neumann et al., 2001). Despite the lack of co-evolution between host and parasite, European honeybees also encapsulate small hive beetles (Breed, 2003; Ellis et al., 2003b) suggesting that encapsulation is part of the general alternative defence of honeybee colonies (Breed et al., 2004).

Recent evidence suggests that small hive beetles also parasitise colonies of other social bees. In fact, small hive beetles have been found infesting commercial bumblebee colonies, Bombus impatiens, in the field (Spiewok and Neumann, 2006b) and in greenhouses (Hoffmann et al., 2008) in North America. Natural small hive beetle infestations were reported in colonies of stingless bees, Dactylurina staudingerii, in West Africa (Mutsaers, 2006) and small hive beetle larvae were also observed in a T. colony that had recently died (Anne Dollin, personal observations) in Australia. Odour cues from stored nest products could attract host-searching adult small hive beetles (Torto et al., 2005; Torto et al., 2007). We therefore expect colonies of stingless bees to be attractive to small hive beetles and, possibly, suitable for their reproduction. Analogous to honeybees, stingless bees also use batumen (a mixture of wax, plant resins and mud) to seal nest cavities as a defence against intruders (Michener, 1961). However, because bees need to enter and exit freely, this strategy still leaves nest entrances vulnerable to direct intrusions and as with honey bees, we expect stingless bees to use alternative defence mechanisms. Our aim for this experiment was to observe the defence behaviour of T. against intruding adult small hive beetles.

9.2 Materials and methods

Laboratory-reared (Muerrle and Neumann, 2004) adult small hive beetles with BaSO4-marked elytra, were introduced to the entrances of five T. hives (N=10 beetles per hive) via a transparent plastic observation tube (Neumann et al., 2001; Hoffmann et al., 2008) and beetle-bee interactions at hive entrances were visually observed. To monitor movements of beetles that managed to bypass entrance guards, hives were scanned at 5 min intervals for 90 min in a human body scanner (General Electric HiSpeed 64 Slice, General Electric Company) (Greco et al., 2006). The methods described by Greco et al. are collectively termed Diagnostic Radioentomology (DR) in this thesis. Beetle distributions within hives were assessed using BeeView 3D rendering software (Disect Systems Ltd; Suffolk, UK). Two dimensional images were created to enable precise measurement of small hive beetle positions and 3D images were generated to provide spatial representation of small hive beetles with respect to hive structures. One hive was randomly selected after scanning and snaps frozen with liquid N_2 for visual screening to compare position and status of small hive beetles with respect to scanned images. A linear mixed model in a block design was applied, with "colonies" as 5 random blocks and "time" as a fixed factor with 10 observations of distances for each time point, to assess any "colony" or "time" effects (it was not possible to fit a 'repeated measurements' model because beetles were not identified as individuals):

$Y_{ijk} = \mu + C_i + t_j + \varepsilon_{ijk}$ where,
Y_{ijk} was k^{th} distance measured in colony i at time j
μ was global mean
C_i was effect of colony I, I = 1,2,…,5 (random)
tj was effect of time j, j = 0, 5, 10 (fixed)
ε_{ijk} was the random error of the k^{th} observation in colony i at time j, k = 1,2,…,10
$C_i \sim N(0, \sigma^2)$
$\varepsilon_{ijk} \sim N(0, \sigma^2)$.

A model of diffusion "random walk" was also tested to assess whether SHB were prevented from normal random walk distributions. If we assume the beetles to move randomly and free of attacks then the model of "random walk" can be helpful for modelling the distribution of measured distances at a given time. This is equivalent to the process of diffusion of a solute in a solvent and can be checked statistically by a Chi-square goodness-of-fit test with the normal distribution of the measured distances. For all statistical analyses we used SYSTAT 12 software (Cranes Software International Ltd.).

9.3 Results

Upon introduction of small hive beetles, visual observations through the transparent plastic tubes confirmed that bees from all T. *carbonaria* hives immediately attacked and coated beetles with batumen at hive entrances. The vigorous attacks by workers (Fig. 14a) caused most beetles to remain motionless, with their heads tucked underneath the pronotum and legs and antennae pressed tightly to the body (= turtle defence posture). When not attacked, beetles were observed progressing further into the hive. However, most T. bees continuously attacked the small hive beetles, thereby keeping them in the turtle defence posture until mummified (Fig. 14b). Although six small hive beetles did not manage to progress into the hives and were mummified on the spot (less than 5 mm from hive entrance), others were able to progress further.

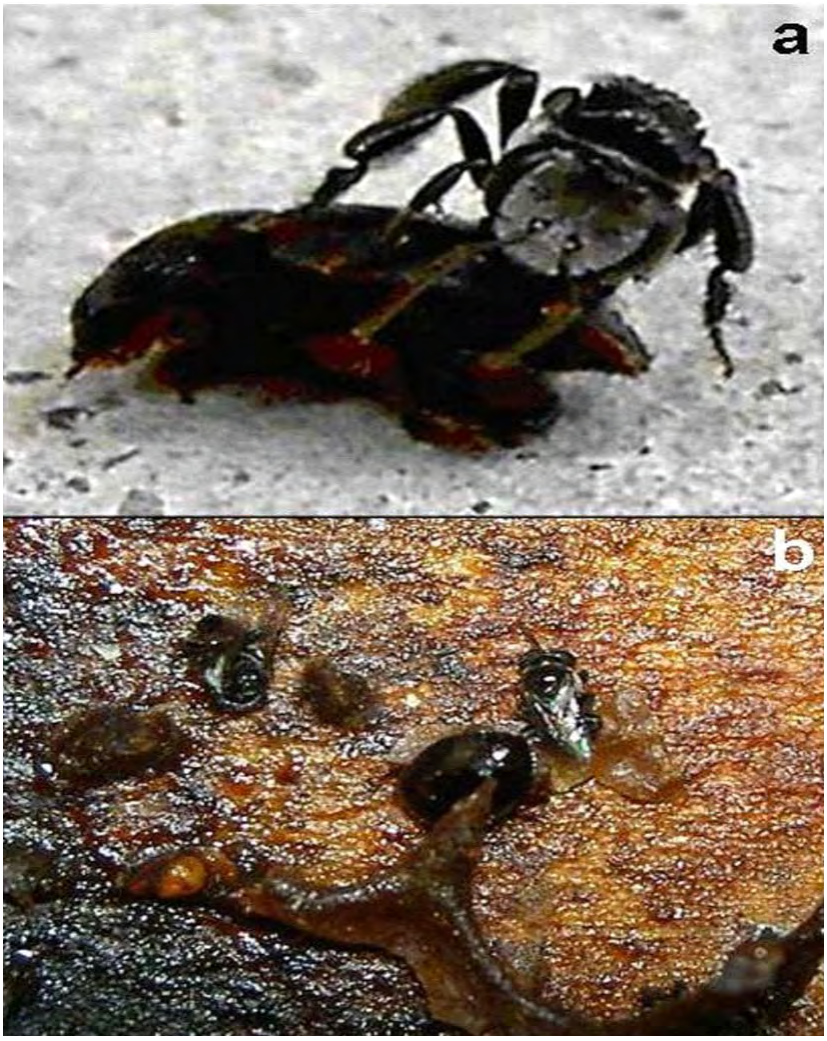


Figure 14: A T. worker mummifies a live small hive beetle by gluing bits of batumen on the beetle's elytra and legs (a) and visual confirmation of a mummified beetle on the floor of a T. hive (b).

In one hive, two small hive beetles reached a distance of 170 mm from the hive entrance, just beneath the brood (Fig. 15a). All advancements by beetles ceased within 10 min of their introduction into the hive (Fig. 15b) and (Fig. 16). After 90 min, mean distance from hive entrances was 60.40 mm S/D 49.55, median distance was 65 mm, maximum distance was 170 mm and minimum distance was 2 mm (N = 950).

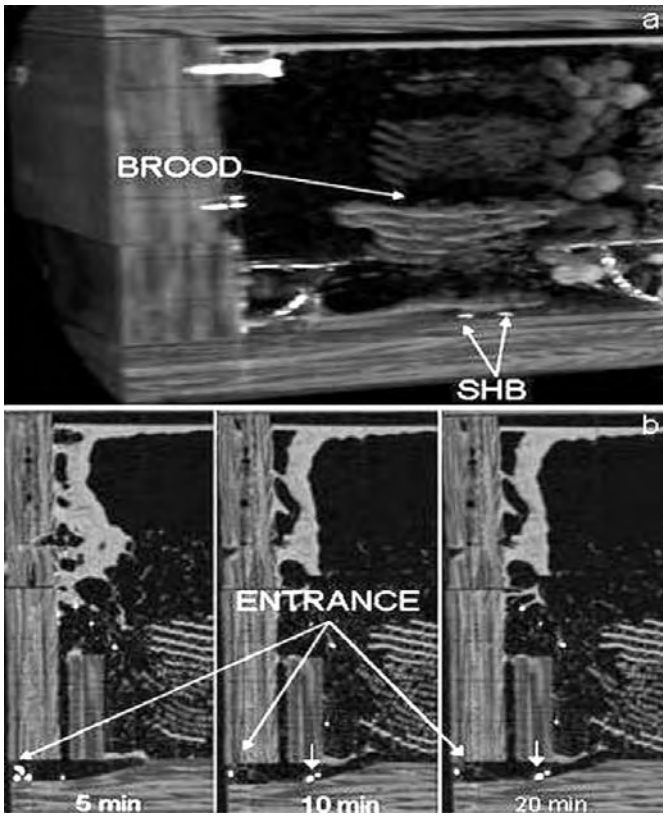


Figure 15: Live mummification of adult small hive beetles in T. *carbonaria* hives visualised by DR: (a) 3D, DR image of T. *carbonaria* brood (single arrow) and two small hive beetles below brood (double arrows); (b) 2D, DR image of small hive beetles (short arrows) in entrance of T. *carbonaria* hive demonstrating no change in position after 10 min.

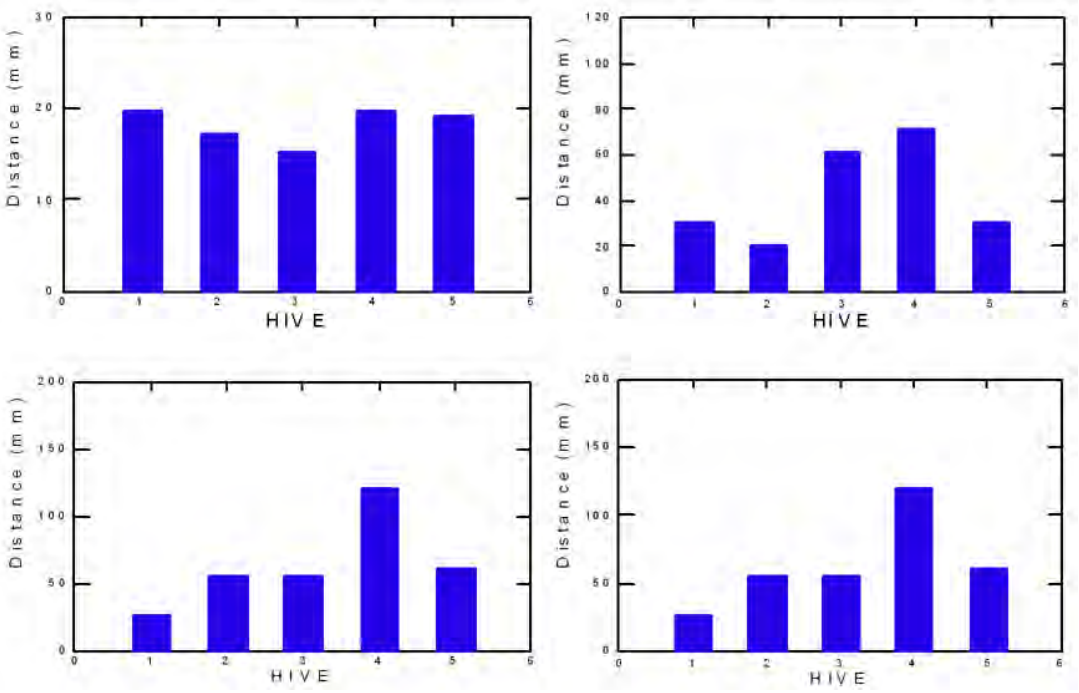


Figure 16: Four bar graphs showing mean distances travelled, from hive entrances, by adult small hive beetles at 0, 5 10 and 90 min after introduction to T. *carbonaria* hives. The 10 min and 90 min graphs are identical because there was no change in distance travelled after 10 min (N = 150).

Dissection of the snap frozen hive confirmed the positions and batumen coatings "pseudo-mummification" of small hive beetles which corresponded with previously scanned images (Fig. 17).

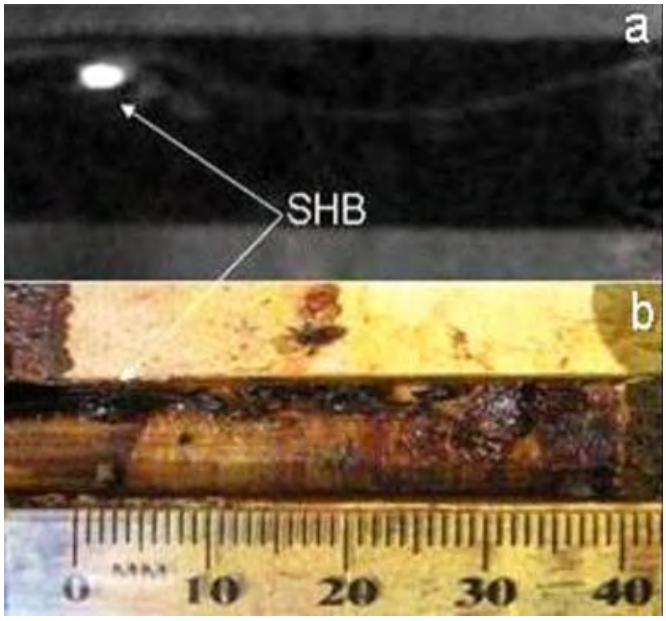


Figure 17: 2D, DR image, taken 90min after introduction to the hive, of an adult small hive beetle which has been mummified by guards of T. *carbonaria* (a) and visual confirmation of the beetle's position and evidence of the batumen coating, applied by guard bees, which prevented further movements by the beetle (b).

Four beetles were visualised at hive entrance (Fig. 18) prior to freezing.

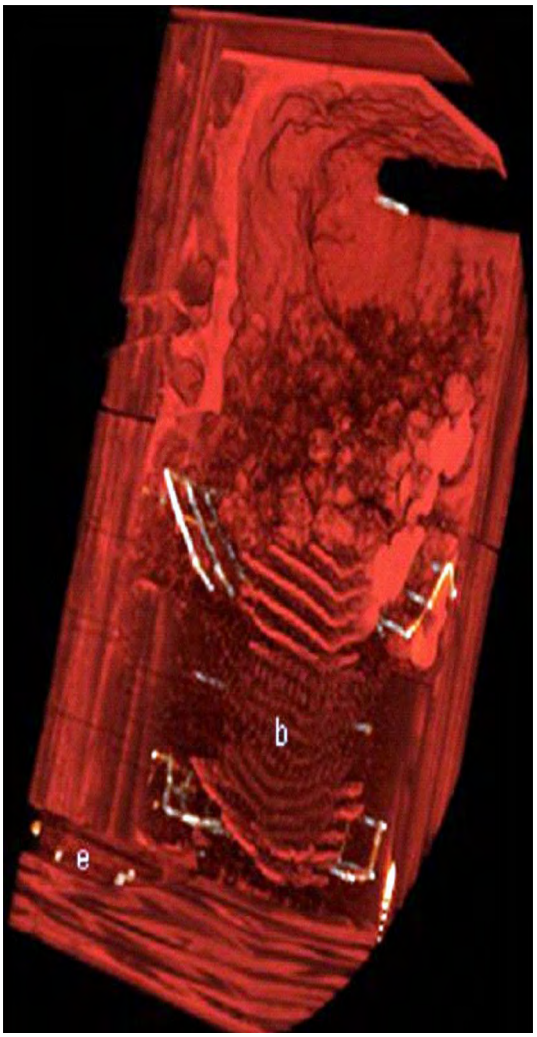


Figure 18: A 3D pseudocolour DR representation of a T. *carbonaria* hive, detailing brood (b) and live mummified small hive beetles (four white oval bodies) in entrance (e).

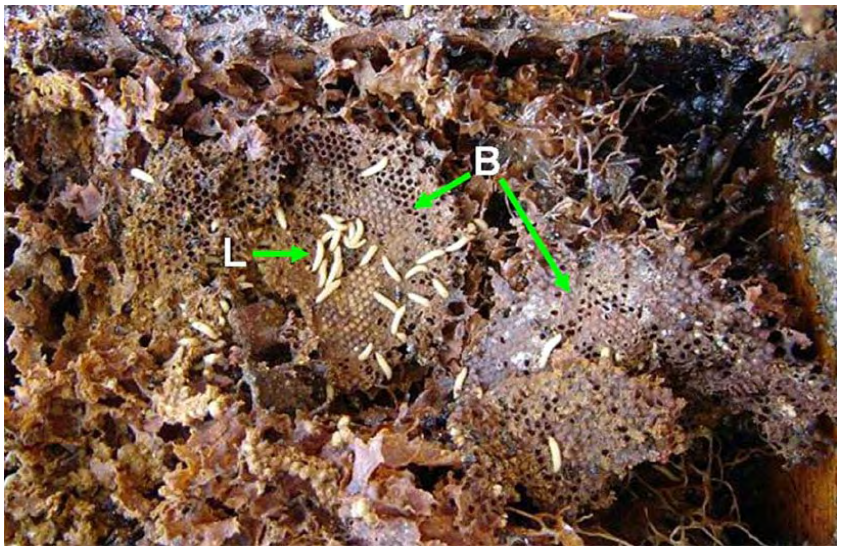


Figure 19: Photograph of a T. *carbonaria* hive invaded by reproducing small hive beetles, detailing brood (B) and small hive beetle larvae (L). The hive became vulnerable to invasion after being weakened as a result of extreme ambient temperature (48° C).

The linear mixed model showed that there was a significant "time" and "colony" effect on beetle distribution, $p < 0.05$ and < 0.001 respectively. Beetles travelled furthest between time 0 and 5 min and travelled least in colony 1 and furthest in colony 4. At "time" 0 to 5 min the model for diffusion or "random walk" was accepted, $Chi2 = 4.12$, $p = 0.53$ (5 min), $df = 5$, showing that beetles were able to disperse between 0 and 5 min. At "time" 10 min the model for diffusion or "random walk" was rejected, $Chi2 = 24.11$, $p < 0.001$, showing that beetles were unable to disperse freely between 5 and 10 min.

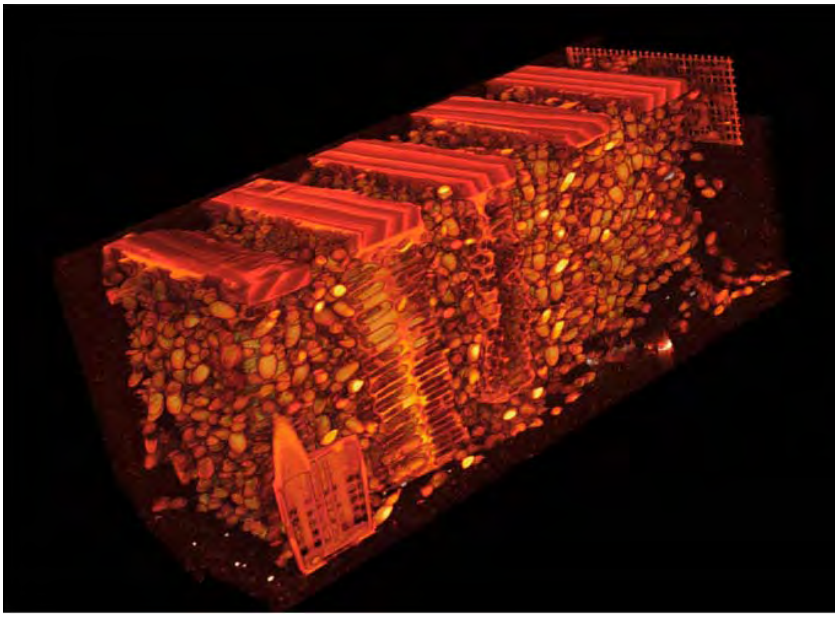


Figure 20: A 3D, DR image of an Apis mellifera colony with adult small hive beetles at the entrance of the hive (White oval shapes, bottom left)

9.4 Discussion

When colonies of social bees are invaded by nest parasites which are difficult for the host to kill or eject (Fig. 20), the host colony faces a dilemma. Successful parasite reproduction must be prevented but direct physical attacks alone are not always sufficient to kill defensive opponents like adult small hive beetles (Currie et al., 1992; Neumann et al., 2001). The encapsulation process of adult small hive beetles in honeybee colonies combines parasite harassment and guarding which usually lasts 1-4 d (Neumann et al., 2001). Beetles mimic worker bee begging behaviour and are fed by worker bees (Ellis et al., 2002), thus allowing enough time for beetle mating to occur (Ellis et al., 2002). The data show that T. *carbonaria* prevented beetles advancements within 10 minutes of introduction by gluing them with batumen (pseudo-mummification) which is an effective alternative defence mechanism to prevent successful parasite reproduction. Although observations by Dollin suggest that native nititulid beetles found in T. *carbonaria* hives do not normally harm the colony, pseudo-mummification may have evolved to prevent these beetles from reproducing in the nest. The beetles are usually found in low numbers, unless the colony has been weakened by other causes such as overheating or insecticide exposure. Brachypeplus macleayi was identified in a Queensland T. *carbonaria* hive in 2002 (Pers comm. A. Dollin). Other nititulid species that have been reported in Australian Trigona nests are B. auritus and B. basalis in Sydney, NSW (Lea, 1910), Carpophilus planatus (Lea, 1912), and B. planus and B. meyricki (Rayment, 1935) in various regions throughout Australia. While social encapsulation of small intruders in wax or propolis confinements has been described from Bombus and Apis (Michener, 1974) and stingless bees coating intruders with resin has been reported (Kerr and Lello, 1962; Nates and Cepeda, 1983; Roubik, 1989; Betz and Koelsch 2004; Lehmberg et al., 2008), to our knowledge, this is the first report of live pseudo-mummification of nest intruders in colonies of social bees.

Using DR, the experiment showed that prevention of small hive beetle advancements by T. *carbonaria* takes as little as 10 min (Fig. 15b), suggesting that pseudo-mummification can be more effective than social encapsulation by honeybees. When small hive beetles adopt the turtle defence posture, most of the honeybee guards leave the beetles, which then scurry into hiding. In contrast, most T. *carbonaria* bees continuously attack the small hive beetles, thereby keeping them in the turtle defence posture. The findings from this experiment suggest that many beetles are immobilised between 5 and 10 min thus they are unable to move unhindered according to a model assuming random walk. This enables workers to mummify the beetles alive with batumen whilst they remain motionless up to 90 min after introduction. Therefore, it appears that the combination of continuous attacks and quick recruitment of mummifying bees underlies this efficient alternative defence mechanism of T. *carbonaria*. There have, however, been reports of heat-stressed T. *carbonaria* colonies being destroyed by small hive beetles in Australia (personal observations), suggesting that this invasive species may still pose some threat to native pollinators (Fig. 19).

In conclusion, single bees, are not able to kill or eject beetle parasites alone. Only a team with individuals performing specific tasks (e.g., wrestling or gluing in the case of live mummification) can overcome parasite advancements. Live mummification of small hive beetles by stingless bees has probably evolved as an alternative defence mechanism to prevent successful reproduction of nest parasites. Using DR as a non-invasive method for observing the interactions between beetles and bees, I was able to demonstrate that the strategy is clearly effective, because small hive beetles are quickly immobilised. Once the beetles are immobilised they are unable to feed or reproduce. This seems especially important in light of the high reproductive potential of small hive beetles. The convergent evolution of live mummification of nest parasites in stingless bees and social encapsulation in honeybees is good example of evolution between insect societies and their parasites. The findings and the novel methods from this experiment have created great interest in the scientific community and has been published in Nature Precedings, Naturwissenschaften, BBC Nature News, Scientific American and New Scientist during 2009.

10 Diagnostic Radioentomology used for studying external and internal morphology of bees.

10.1 Introduction

Traditionally, the morphological classification of bees has been conducted with the aid of dissecting light microscopy (Michener, 1960; Wille, 1979) to visualise structures such as wing venation, tarsal segments and other morphology. In more recent times, detailed information for bee classification based on their external and internal morphology has been obtained with the use of scanning electron microscopy (SEM) (Serrao and da Cruz-Landim 2000; Serrao, 2001; Serrao, 2005) and transmission electron microscopy (TEM) (Araujo et al., 2005). The data from Serrao (2001) suggest that the Meliponini and Apini are a monophyletic group and that Bombini and Euglossini are also a monophyletic group. Roig-Alsina and Michener (1993) suggested that all stingless bees belong to one tribe, the Meliponini. This hypothesis has been supported by Serrao (2001) who looked at the proventricular morphology of bees and determined that the Euglossini and Bombini have long columnar proventricular plates, the Apini have triangular apices to their plates whilst the Meliponini have slender and elongated plates. Thus, the proventriculus is an important diagnostic structure for bee taxonomy.

Although SEM and TEM provide the highest level of detail, sample preparations are laborious, time consuming, invasive and destructive for each investigation (Serrao, 2001). X-ray computerised tomography (CT) has previously been adopted to visualise macroscopic characteristics of insects. MicroCT is now emerging as a new method for the non-invasive imaging of insects at the microscopic level (Hornschemeyer et al., 2002; Johnson et al., 2004). The basic principles of MicroCT are similar to those used in medical CT scanners; however, with MicroCT, it is now possible to achieve a resolution down to a few micrometers (Bettuzzi et al., 2004; Feeney et al., 2006). MicroCT, including synchrotron MicroCT (see Appendix 2), along with NanoCT and MacroCT, which is collectively described as DR are emerging technologies (Tollner, 1991; Fuchs et al., 2004; Greco et al., 2005; Greco et al. 2006) that are proving useful for entomologists. Below, the use of MicroCT for non-invasively and non-destructively assessing the morphology of an Australian stingless bee, T. *carbonaria*, with particular focus on the proventricular plates, is described.

10.2 Materials and methods

10.2.1 Sample preparation

For MicroCT, a worker of the bee species T. *carbonaria* was placed in a 0° C freezer for 15 min. T. *carbonaria* was selected for examination as it is relatively common and well studied (Michener, 1961; 1974; Heard, 1988a; 1988b, 1994, 1999, 2001; Bartareau, 1996; Dollin et al., 1997; Dollin, 1998a, 1998b, 1999, 2000a, 2000b, 2000c, 2000d; Amano et al., 2000; Amano, 2002, 2004, 2005; Greco et al., 2005). The dead bee was placed in a 15 mm length of polyethylene drinking straw (internal diameter 3 mm). The two ends of the straw were sealed with polystyrene foam to keep the bee in position whilst being scanned. For light microscopy, a worker bee was dissected and viewed using a Leica MZ12 stereomicroscope, Leica Microsystems GmbH Ernst-Leitz-Strasse 17-37 35578 Wetzlar. For comparison of internal abdominal morphology, an individual of Amegilla sp., which was pinned for a museum's bee collection six years ago, was also scanned using the same preparation as above; however, the specimen was not dissected afterwards, to preserve integrity of the specimen.

10.2.2 MicroCT scanning

Scans were performed with a Skyscan 1172 high-resolution MicroCT system. This system has a sealed, microfocus x-ray tube with a 5 µm spotsize. The x-rays were produced by exposing the anode to 40 kV at 100 µA. Prior to scanning, the sample was placed on the pedestal between the x-ray source and the CCD detector. After positioning the sample, 600 2D x-ray images over 180° were captured by exposing the sample and then rotating it to the next exposure position with a slice-to-slice rotation distance of 2 µm, and a total acquisition time of approximately 60 min: each 2D image represents one slice. The scanner software then converted each slice to axial orientation and created 998 bitmap images (16 bit grey scale) which were stored for 2D viewing and 3D reconstruction as a 983Mb dataset.

10.2.3 Image processing

The 3D reconstruction and analysis of the x-ray images were performed (Greco et al., 2005). Using multi-planar reformatting (MPR) algorithms these software programs enabled the 2D x-ray images to be reformatted into a 3D model. The model was further manipulated by adjusting window levels (WL) and window widths (WW) to enhance visualisation of the morphological structures. Greatest visual enhancement was achieved at WL 150 and WW 158. For greater flexibility in software usage, VG STUDIO MAX was used while at the scanner workstation and BeeView was used remotely on a laptop computer. The sample was viewed from many angles along randomly selected axes. Sections of the model were also removed along cutting planes which were positioned by using the computer mouse. The cutting planes acted like a virtual scalpel enabling visualisation of the bee's internal morphology including fine detail of the bee's proventricular plates. Image magnification was performed when greater detail was required.

10.3 Results

Although the resolution of MicroCT is not as fine as SEM, the images presented demonstrate that the technique is useful for viewing and assessing the external and internal morphology of bees accurately and non-invasively. Gross external morphological features such as the articulations of the coxae, trochanters, tibiae and tarsi of each leg including broadened hind basitarsi (Fig. 21) could be seen.

Image magnification revealed further detail such as antennal scapes and the various parts of the mouth including the proboscis and labium (Fig. 22). However, the individual facets of the eye were barely discernable and MicroCT did not reveal fine details of hairs on the body or legs.

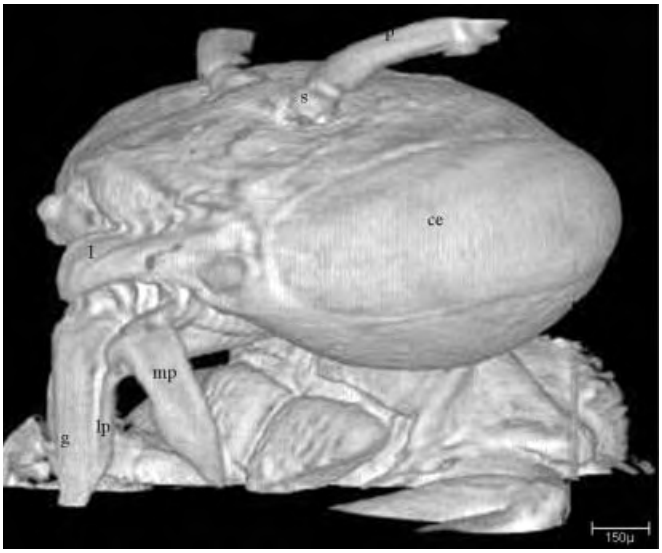


Figure 22: 3D reconstruction of the head of a worker of T. *carbonaria* showing details of external morphology: antennal pedicle (p), antennal scape (s), compound eye (ce), labrum (l), maxillary palpus (mp), labial palpus (lp) and glossa (g).

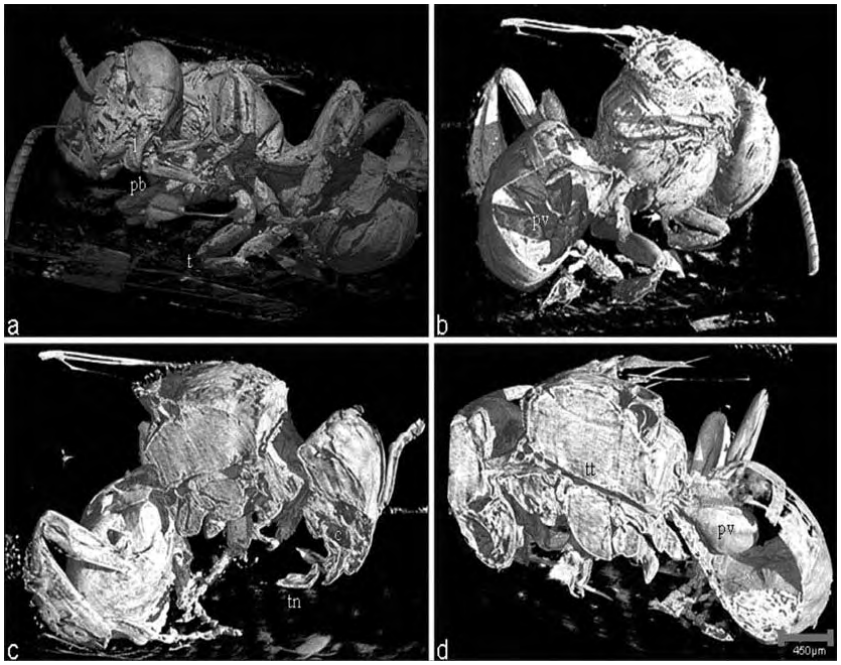


Figure 21: MicroCT images of a worker bee of T. *carbonaria*: a. ventral aspect showing tarsi (t), proboscis (pb) and labium (l); b. postero-dorsal view with virtual dissection of abdomen and proventriculus (pv); c. right lateral view showing cibarium (c) and longitudinal section of the tongue (tn); and d. left lateral view showing lateral longitudinal tracheal trunk (tt) and proventriculus (pv).

The two software programs (VG STUDIO MAX V1.2 and BeeView 3D) were user friendly and provided powerful image processing, including the ability for virtual dissection. Unfortunately, many soft tissues did not produce sufficient differentiation, and it was not possible to discern structures such as ovaries and details of the hind gut. Attempts were made to use medical grade x-ray contrast agent to outline these soft tissue regions; however, the results were poor and more research into the use of contrast agents will be required to improve soft tissue differentiation in insects using MicroCT. However, some internal structures could be clearly visualised. For example, Figure 20d shows the lateral longitudinal tracheal trunk. In particular, because the proventriculus and proventricular plates are lined with cuticle, these structures were better differentiated and were clearly visualised and discernible for diagnostic purposes (Figs 23 & 24). Figure 22b shows the slender and elongated outlines of the basal plates that line the proventriculus and which form the leaflets of proventricular valve. The leaflets of these plates are covered in hairs (Serrao, 2005), but these were not visible using MicroCT. Figures 23c and d show the proventriculus at the distal part of the crop and the cross-shaped leaflets of the proventricular valve forming the opening into the proventriculus.

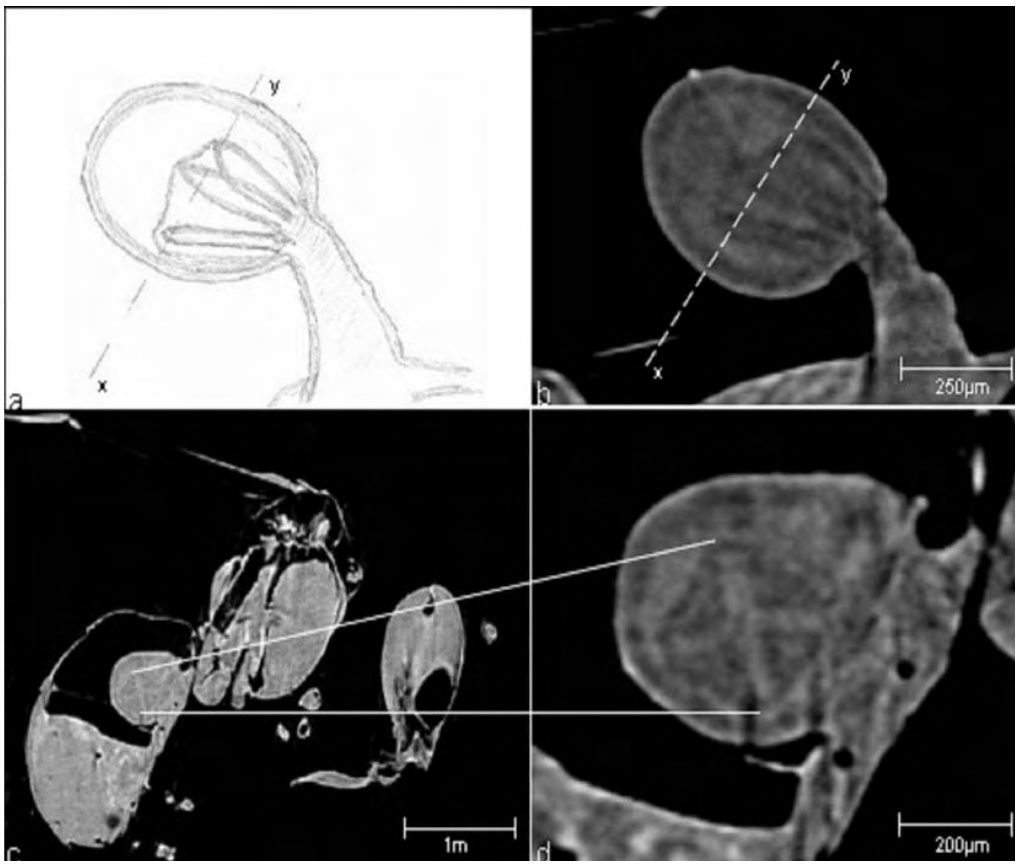


Figure 23: Details of the proventriculus, proventricular valve and elongated proventricular plates of T. *carbonaria*: a. line diagram of the image in Fig. 3b; b. cross section of proventriculus showing characteristic slender, elongated, Meliponini-shaped plates using MicroCT; c. MicroCT of proventricular valve showing the four plates in closed position; and d. magnified image after using electronic dissection at cutting plane x-y.

Figure 24 shows images of the proventriculus using dissection light microscopy and MicroCT. The image acquired from light microscopy resulted from dissections of six worker bees which were destroyed in the process whereas the images acquired during MicroCT required just one bee which was undamaged. The characteristic features of the proventricular plates of Meliponini could thus be quickly and easily identified non-invasively.

Figure 25 demonstrates the type of morphological variations that can be identified in more detailed future studies using MicroCT. The internal morphology of the fifth abdominal sternite of these very different bee species is similar, but the T. *carbonaria* sternite shows a pronounced notch along its lateral margin which is absent in the Amegilla species.

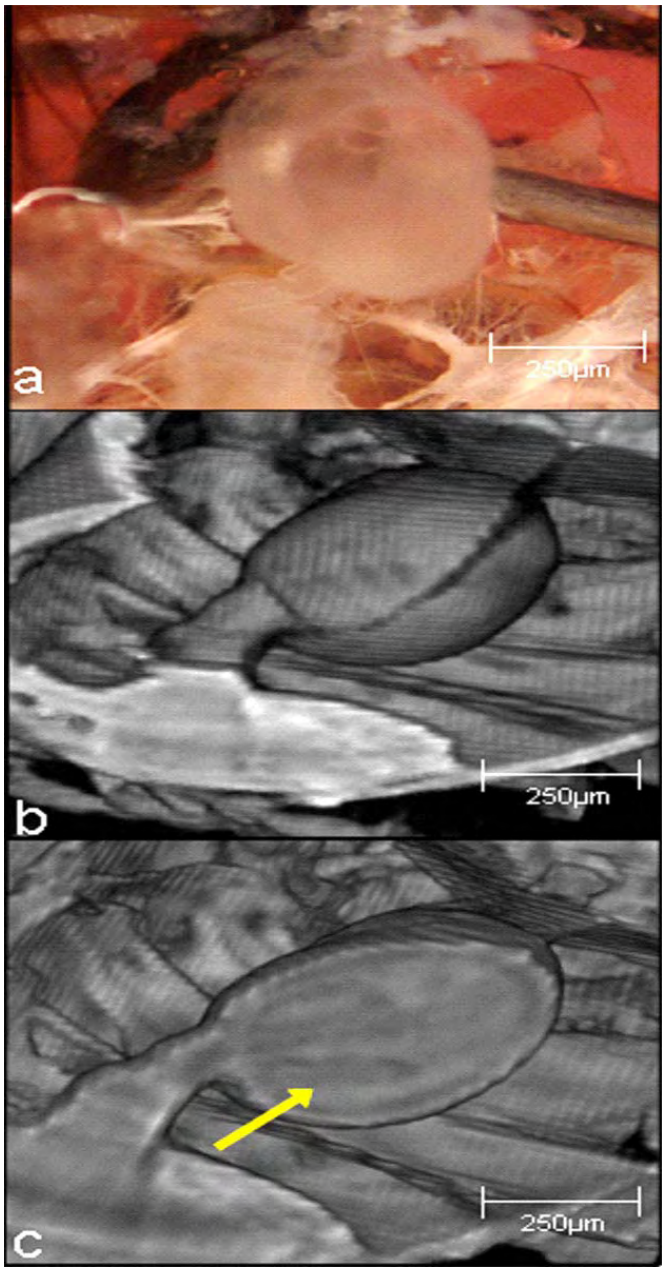


Figure 24: T. *carbonaria* proventriculus anatomy: Dissection under light microscopy showing the proventriculus a. MicroCT images of the proventriculus showing a 3D reconstruction of proventriculus b and details of the slender, elongated, proventricular plates (arrowed) c.

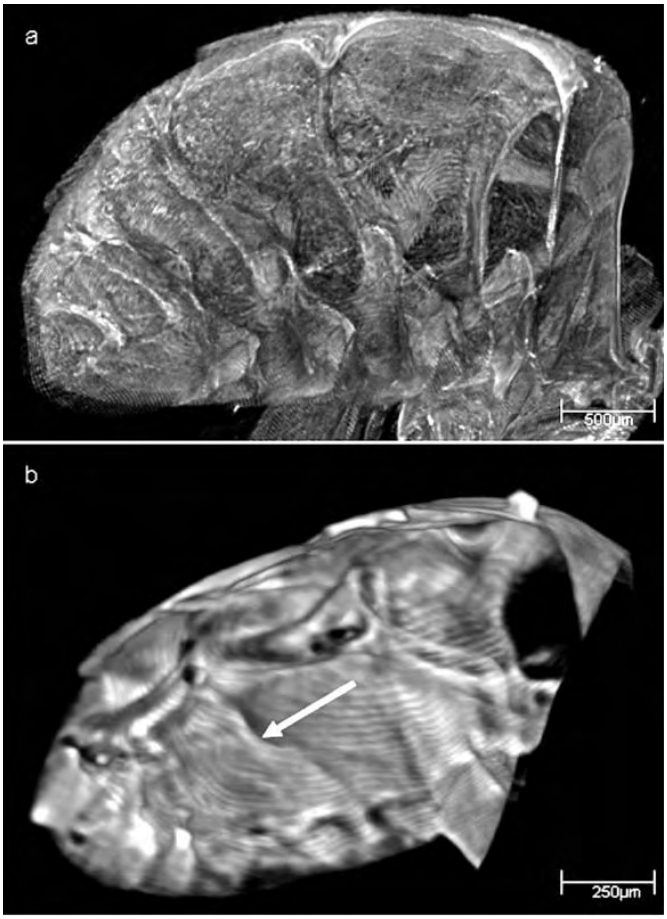


Figure 25: 3D MicroCT images showing internal abdominal morphology of two very different bee species. a. A six year old Amegilla sp. museum specimen demonstrating internal morphology of sternites and b. a fresh specimen of T. *carbonaria* demonstrating similar morphology but with a notch evident on the lateral margin of the fifth sternite (arrow).

11 Future Directions

10.4 Discussion

This new approach is not destructive to specimens, unlike the traditional methods of dissection light microscopy, SEM or TEM (Serrao, 2001; Araujo et al., 2005; Serrao, 2005). The procedures outlined in this paper allowed the visualisation of internal and external morphological features to be carried out without lengthy and laborious dissections or preparations required by these techniques. Although the samples in this study were dead, the scans could also be performed on live, immobilised insects without harming them. The scans take approximately 1 h and once the images are saved as electronic datasets in bmp, jpeg or DICOM formats, the 3D reconstruction and virtual dissecting can be repeated indefinitely. In addition, virtual dissection can be performed in planes at infinite angles on the same specimen which can not be done when using SEM, TEM or light microscopy, thus making morphological analysis much less laborious for the researcher. The researcher can also electronically store each dataset and return to the same sample at a later stage to review the same morphology or explore new regions within the sample.

The non-destructive nature of MicroCT will be invaluable for rare or precious specimens, such as those in museums, where there may be only one or two specimens available for examination, or for scanning holotypes. MicroCT also has the advantage of being able to scan insects in amber or as fossils without damaging the specimens. This will have important uses for non-invasive phylogenetic studies on ancient insects such as bees in amber.

The findings from this experiment were published in the Journal for Apicultural Research which is a peer reviewed internationally recognised journal. Greco et al received the Dr Eva Crane Award for the most original and ground breaking research during 2008 from The International Bee Research Association (IBRA). The recent refinements in NanoCT imaging will allow image resolution to move closer to the detail revealed by SEM, making DR the technique of choice for entomologists in the future.

As with the biotic and abiotic requirements of bees, there has been limited research conducted on pests and diseases that effect the long-term survival of bee colonies. Conducting future investigations, such as those by Stow et al. (2007) on antimicrobial activity of bee secretions, could provide valuable insight into methods for promoting long-term colony survival under pollination service regimes. Conducting experiments on the impacts of pests and diseases on the population dynamics of managed colonies would also provide useful information for developing strategies to prevent colony decline.

11.1 Prospects for diagnostic radioentomology

The invasive processes of opening hives for observations, described above kills many bees. Thus, these novel methods for non-invasively monitoring colony health in bees using MacroCT were developed. Also, because the health of individual bees impacts on the health of the colony, I explored the use of MicroCT for assessing internal and external morphology of individual bees and collectively termed the methods I developed diagnostic radioentomology (DR).

11.2 MacroCT

As detailed above and in Greco et al. (2005), MacroCT utilises human body CT scanners and is extremely useful for assessing large samples such as beehives. The methods I have developed using MacroCT are ideal for experiments on insect behaviour, assessing population dynamics and colony health in bees and other social insects. After the initial scans are performed and a digital dataset is generated, the process then becomes portable and can be used to assess hive components separately to monitor colony health and for observing the behaviour of individuals in a colony (Greco et al., 2009) on laptop or desktop computers (Fig. 26).

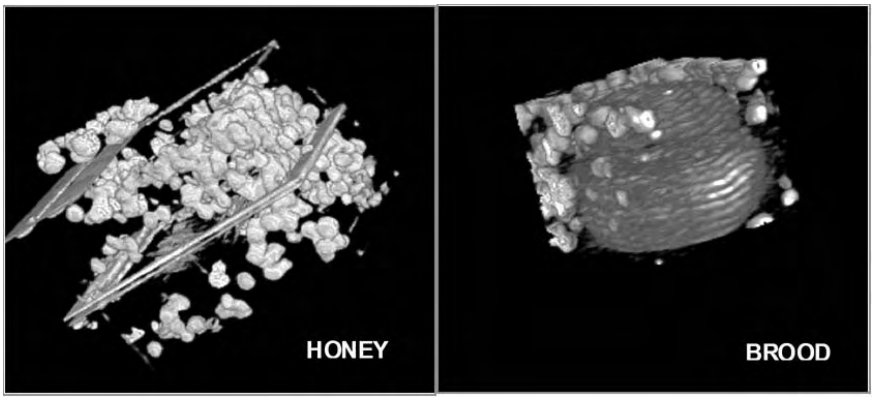


Figure 26: Two 3D images of the same TC hive containing a colony of T. *carbonaria*. Both images were produced from the same digital dataset enabling hive components to be assessed separately. For non-invasive assessment of individual hive components, all were digitally removed except for honey (a) and brood (b).

Similar to other imaging instruments such as SEM's or confocal microscopes, access to MacroCT scanners needs to scheduled and scans currently cost approximately $AUD 100 per hour and, with appropriate planning, DR could be an extremely useful method for researchers who require non-invasive approaches to their experiments.

11.3 MicroCT

As detailed above and in Greco et al. (2008), MicroCT utilises bench-top CT scanners to produce digital datasets of individual bees. MicroCT is extremely useful for assessing small samples such as individual insects. The methods I have developed using MicroCT are ideal for evaluating external and internal morphology of insects such as bees non-invasively. I have commenced experiments on live bees; however, scan time can take up to 1 h so I am further developing methods for faster scans and for immobilising live insects by cooling and adding vaporised, frozen CO2.

11.4 Other techniques

The new DR methods I have been developing are based on medical imaging modalities and, to date, CT has proved to be the most useful for studying insects because of the high resolution of 0.3 mm and 5 µm for MacroCT and MicroCT, respectively, and the short scan times for both. I have investigated the possibility of using NanoCT because the resolution is approximately 100nm to 200nm however scanning times are extremely long (in excess of 3 h) and sample size is limited to small insects (3mm - 4mm) I have tested other medical imaging modalities such as magnetic resonance imaging (MRI) and diagnostic ultrasound (U/S) on bees. Although I have had limited success with these modalities, they also have great potential for future DR studies. MRI offers better tissue differentiation but has poorer image resolution than CT and scans can take as long as 3 h. U/S is currently limited to low resolution imaging of internal (bee) structures, because the high frequency sound waves used in U/S imaging do not travel though air. This means that the U/S probe needs to be placed on the bee's cuticle to enable internal structures to be visualised.

DR is not limited to bees and can be used for non-invasive assessment of most insects. Below, I present several examples of DR images of insect nests including ants (Fig. 27), to demonstrate its usefulness.

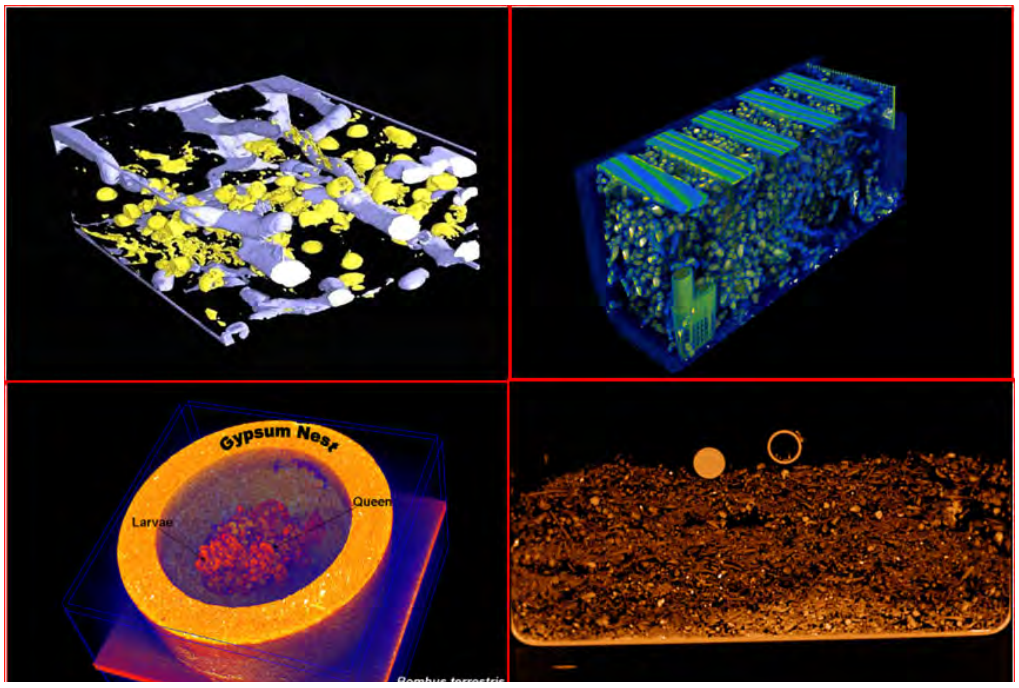


Figure 27: DR images of three genera of bees and one ant genus. An experimental soil nest containing adults and brood of the solitary fossorial bee A. holmesi (a), an experimental hive containing a colony of A. mellifera (b), an experimental gypsum nest containing a colony of B. terrestris (c) and an experimental detritus nest containing a colony of a terrestrial Formica species (d).

11.5 Work in progress

I have utilised the results from my doctoral studies to further use and develop the techniques associated with diagnostic radioentomology (DR). I have collaborated with many institutions whose researchers are recognising the potential for DR. Some of the institutes that have collaborated to develop my new methods for non-invasive examinations of insects include: Agroscope Liebefeld Posieux (ALP), Paul Scherer Institute (PSI), University of Zurich (VETSUISSE ZURICH), University of Bern (VETSUISSE BERN & UNIB), European Synchrotron Radiation Facility (ESRF) and Elettra Synchrotron Light Source (ELETTRA). Initial work with the Swiss Bee Research Institute and the University of Bern has helped to develop alternative methods for monitoring population dynamics in A. mellifera colonies (see Appendix 3) using DR. Together, we have been refining methods for assessing population dynamics and improving software programs for achieving greater accuracy when quantifying hive components. The new approach is non-invasive and will eventually replace the globally accepted "Liebefeld Method" for population dynamics assessment , because of its increased accuracy in quantifying bee numbers and hive components.

Preliminary DR work at PSI, VETSUISSE, University of Bern and ELETTRA has demonstrated the usefulness of DR (see appendix 4) for assessing population dynamics and bee behaviour in managed A. mellifera colonies. It has also been useful for assessing internal morphology of V. destructor, T. *carbonaria* and an ancient stingless bee trapped in amber.

DR is particularly useful for precious specimens, such as those trapped in amber. Mineral, plant and animal inclusions in amber can shed light on the prevailing conditions of our planet millions of years ago. Many amber inclusions are difficult or impossible to view under light microscopy because amber is often opaque and clear amber diffracts light which degrades the image. A DR study I conducted (Fig. 28) revealed internal morphology of the amber bee. The internal tissue was too desiccated to compare with modern species; however, many external morphological features such as compound eyes, ocelli, mandibles, legs, wings etc. were clearly visible for comparisons. The presence of a double spur on the front mid tibial segment, for example, suggests that the specimen could be a previously undescribed species (D. Roubik, Smithsonian Institute, pers. comm.., 19th Nov 2009).

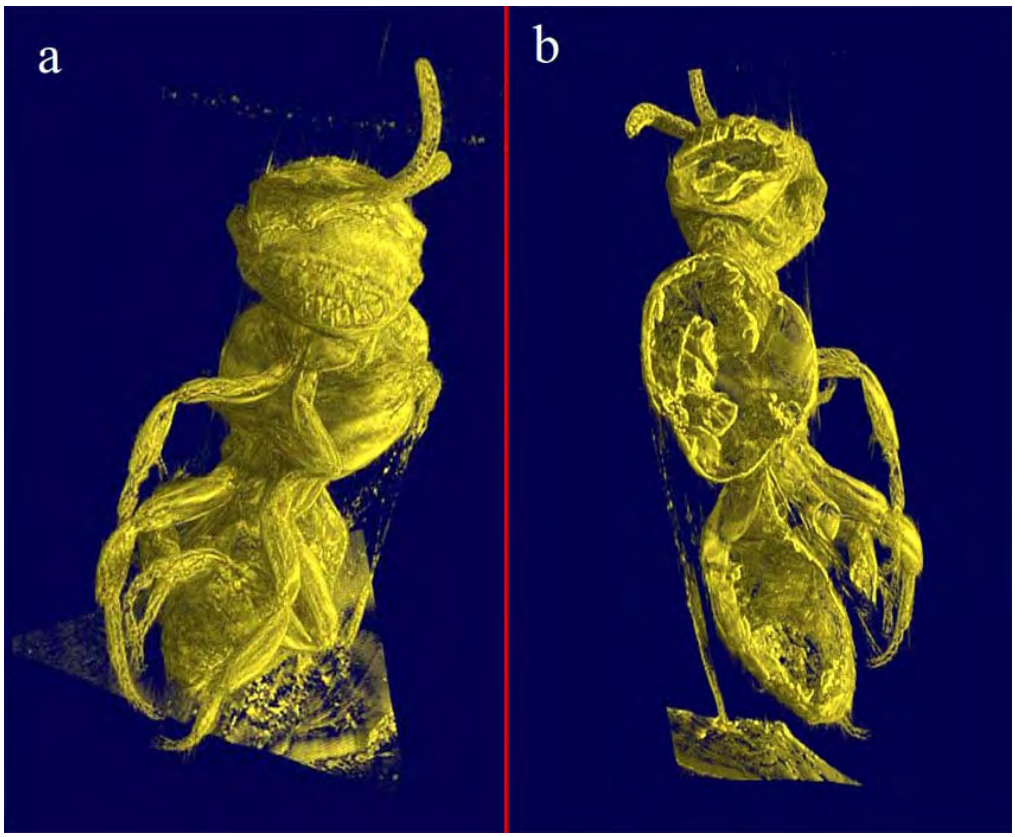


Figure 28: External (a) and internal (b) morphology of an ancient bee trapped in amber. The bee is probably (Proplebeia dominicana Wille and Chandler.; Hymenoptera: Apidae) and is approximately 25 MA; however, the presence of a double spur (arrow) suggests that this specimen might be a previously undescribed species.

The potential for DR as a non-invasive method to study insects is only now starting to be investigated and future research could focus on behaviour experiments, such as feeding experiments with food labelled with radiographic contrast or physiological as well as morphological studies such as tracing labelled food through the insect's gut to the haemolymph. I present such an example in Appendix 3.

11.6 General Conclusion

This research has determined that the sensitivity of the data produced from the new imaging techniques requires further developments to produce more reliable results. The benefits this research brings to apicultural practices to increase the health and long-term survival of bees plus the benefits to the scientific process of observing insects justify continuation of this line of research for the development of improved methods for apiculturalists and scientists.

12 References

Alcock, J., L.W. Simmons and M. Beveridge (2005). Seasonal change in offspring sex and size in Dawson's burrowing bees (Amegilla dawsoni) (Hymenoptera: Anthophorini). Ecological Entomology 30: 247–254.

Amano, K. (2002). Stingless honeybees for Asia's greenhouses. Food and fertilizer technology centre http://www.fftc.agnet.org/library/article/nc138a.html. Retrieved 1 December 2002.

Amano, K. (2004). Attempts to introduce stingless bees for the pollination of crops under greenhouse conditions in Japan. National Institute of Livestock and Grassland Science http://www.agnet.org/library/tb/167. Retrieved 11 April 2007.

Amano, K. (2005). Attempts to introduce stingless bees for the pollination of crops under greenhouse conditions in Japan. Retrieved 11 April 2007.

Amano, K., T. Nemoto and T.A. Heard (2000). What are stingless bees and why and how to use them as crop pollinators. Japan Agricultural Research Quarterly 34(3): 183-190.

Araujo, V.A., U. Zama, H. Dolder and J. Lino-Neto (2005). Morphology and ultrastructure of the spermatozoa of Scaptotrigona xanthotricha Moure (Hymenoptera: Apidae: Meliponini).

Brazilian Journal of Morphological Science 22: 137–141.

Bartareau, T. (1996). Foraging Behaviour of Trigona *carbonaria* (Hymenoptera: Apidae) at Multiple-choice feeding stations. Australian Journal of Zoology 44: 143-153.

Bell, M. C., R. N. Spooner-Hart, et al. (2006). Pollination of Greenhouse Tomatoes by the Australian Bluebanded Bee Amegilla (ZonAmegilla) holmesi (Hymenoptera: Apidae). Journal of Economic Entomology 99(2): 437-442.

Bettuzzi, M.S., F. Casali, S. Cornacchia, M. Rossi, E. Paltrinieri, M.P. Morigi, R. Brancaccio and D. Romani (2004). A new linear array detector for high resolution and low dose digital radiography. Nuclear Instrumentation and Methodology B 213: 227–230.

Betz, O. and G. Koelsch (2004). The role of adhesion in prey capture and predator defence in arthropods. Arthropod Structure and Development 33: 3–30.

Breed, M.D. (2003). Nestmate Recognition Assays as a Tool for Population and Ecological Studies in Eusocial Insects: A Review. Journal of the Kansas Entomological Society. 76: 539-550.

Breed, M.D., Guzman-Novoa E, Hunt GJ (2004) Defensive behaviour of honey bees: Organization, Genetics, and Comparisons with Other Bees. Annual Review of Entomology 49:271–98.

Brothers, D.J. (1999). Phylogeny and evolution of wasps, ants and bees (Hymenoptera, Chrysidoidea, Vespoidea and Apoidea). Zoologica Scripta 28: 233-249.

Cardale, J. (1968). Nests and nesting behaviour in of Amegilla (Amegilla) pulchra (Smith) (Hymenoptera: Apoidea: Anthophorinae). Australian Journal of Zoology 16: 689-707.

CSIRO (1991) The Insects of Australia, 2nd edn. Melbourne University Press, Melbourne, Australia.

Currie, R.W., M.L. Winston and K.N. Slessor (1992). „Effect of Synthetic Queen Mandibular Pheromone Sprays on Honey Bee (Hymenoptera: Apidae) Pollination of Berry Crops." Journal of Economic Entomology 85(4): 1300-1306.

Dainat, B., M. K. Greco and P. Neumann (2009). Spatial Distribution of Honeybees in the Winter Cluster investigated by Diagnostic Radioentomology (DR). 41st International Apimondia Conference Mont Pellier.

Dollin, A (1996a) Introduction to Australian Native Bees. North Richmond, Native Bees of Australia Series, Booklet 1. The Australian Native Bee Research Centre; North Richmond, Australia, 10 pp.
Dollin, A. (1996b). Behaviour of Australian stingless bees. North Richmond, Australian Native Bee Research Centre.

Dollin, A. (1998a) Stingless beekeeping with Trigona in Vietnam. Aussie Bee 7: 7-9.

Dollin, A. (1998b). Stingless beekeeping with Trigona in Vietnam. Aussie Bee. 7: 13.

Dollin, A (1999) A winter's tale… Stingless bees thrive in glasshouse trial. Aussie Bee 12: 8–11.

Dollin, A (2000a). Stingless bees pollinate greenhouse capsicums. Aussie Bee 13: 15.

Dollin, A. (2000b). Choosy Bees Make Prime Pollinators. AUSSIE BEE 13: 14-15.

Dollin, A. (2000c). Stingless Bees Pollinate Greenhouse Capsicums. Aussie Bee 13: 15.

Dollin, A. (2000d). Our Bees In Japan. AUSSIE BEE 15: 16-19.

Dollin, A. (2007). Submission to inquiry into the future development of the Australian honeybee industry. Australian Native Bee Research Centre: 3. North Richmond, NSW Australia.

Dollin, A., L. Dollin and S. Sakagami (1997). Australian Stingless Bees of the Genus Trigona (Hymenoptera: Apidae). Invertebrate Taxonomy 11: 861-896.

Dollin, A. and T.A. Heard (1997) Keeping Australian stingless bees in a log or box. Native Bees of Australia Series, Booklet 5. The Australian Native Bee Research Centre; North Richmond, Australia, 15 pp.

Dollin, A., R. Zabel and J. Zabel (2001).Boxing and Splitting Hives -- A Complete Do it Yourself Guide for Stingless Bee Keepers. Native Bees of Australia Series, Booklet 9. The Australian Native Bee Research Centre; North Richmond, Australia, 19 pp.

Ellis, J.D., H.R. Hepburn, K. Delaplane, P. Neumann and P.J. Elzen (2003a). The effects of adult small hive beetles, Aethina tumida (Coleoptera: nitidulidae), on nests and flight activity of Cape and European honey bees (Apis mellifera). Apidologie 34, 399-408.

Ellis, J.D., H.R. Hepburn, A.M. Ellis and P.J. Elzen (2003b). Social encapsulation of the small hive beetle (Aethina tumida Murray) by European honeybees (Apis mellifera L.). Insectes Sociaux 50:286–291.

Ellis, J.D., C. Pirk, H.R. Hepburn, G. Kastberger and P.J. Elzen (2002). Small hive beetles survive in honeybee prisons by behavioural mimicry. Naturwissenschaften 89: 326-328.

El-Niweiri, M.A.A., M.S. El-Sarrag and P. Neumann (2008) Filling the Sudan gap: the northernmost natural distribution limit of small hive beetles. Journal of Apicultural research 47 (3):183-184.

Elzen P.J., J.R. Baxter, P. Neumann, A. Solbrig, C.W.W. Pirk, H.R. Hepburn, D. Westervelt and C. Randall (2001). Behaviour of African and European subspecies of Apis mellifera toward the small hive beetle, Aethina tumida. Journal of Apicultural Research 40:40-41.

Elzen, P.J., J.R. Baxter, D. Westervelt, C. Randall, K.S. Delaplane, L. Cutts and W.T. Wilson (1999). Field control and biology studies of a new pest species, Aethina tumida Murray (Coleoptera, Nitidulidae) attacking European honey bees in the Western hemisphere, Apidologie 30:361–366.

Elzen, P.J., J.R. Baxter, D. Westervelt, C. Randall and W.T. Wilson (2000) A scientific note on observations of the small hive beetle, Aethina tumida Murray (Coleoptera, Nitidulidae) in Florida, USA. Apidologie 31:593-594.

Engel, M. S. (2000). A New Interpretation of the Oldest Fossil Bee (Hymenoptera: Apidae). AMERICAN MUSEUM NOVITATES 3296: 11.

Feeney, D.S., J.W. Crawford, T. Daniell, P.D. Hallett, N. Nunan, K. Ritz, M. Rivers and I.M. Young (2006). Three-dimensional microorganization of the soil–root–microbe system. Microbiology and Ecology 52: 151–158.

Fuchs, A., A. Schreyer, S. Feuerbach and J. Korb (2004). A new technique for termite monitoring using computer tomography and endoscopy. International Journal of Pest Management 50: 63–66.

Greco M, Spooner-Hart R and Holford P (2005) A new technique for monitoring Trigona *carbonaria* nest contents, brood and activity using X-ray computerised tomography. Journal of Apicultural Research 44: 97–100.

Greco M.K., Bell M, Spooner-Hart R, Holford P (2006) X-ray computerized tomography as a new method for monitoring Amegilla holmesi nest structure, nesting behaviour and adult female activity. Entomologia Experimtalis et Applicata 120:71-76.

Greco, M., Jones, A., Spooner-Hart, R. and Holford, P. 2008 X-ray computerised microtomography (MicroCT): a new technique for assessing external and internal morphology of bees. Journal of Apicultural Research 47: (4) 286-291.

Greco M.K., R. Spooner-Hart and P. Holford (2010a) A stingless bee hive design for a broader climate range Journal of Apicultural Research (with referees).

Greco, M.K., R. Spooner-Hart, A. Beattie and P. Holford (2010b). Australian Stingless Bees Improve Greenhouse Capsicum Production. Journal of Apicultural Research (with referees).

Greco, M. K., R. Spooner-Hart, A. Beattie and P. Holford (2010c). Optimum bee stocking rates for pollination of Greenhouse Capsicum By Trigona *carbonaria*. Journal of Apicultural Research (with referees).

Green, C.L., Franck, P., Oldroyd, B.P. (2001) Characterization of microsatellite loci for Trigona *carbonaria*, a stingless bee endemic to Australia. Molecular Ecology Notes 1: 89–92.

Grimaldi, D. and M. S. Engel (2005). Evolution of the insects., Cambridge University Press, New York.

Haddad N, J. Esser and P. Neumann (2008) Association of Cryptophagus hexagonalis (Coleoptera: Cryptophagidae) with honey bee colonies (Apis mellifera). Journal of Apicultural Research 47:189-190

Harrison RD, Gardner WA, Tollner WE and Kinard DJ (1993) X-ray computed tomography studies of the burrowing behaviour of fourth-instar pecan weevil (Coleoptera: Curculionidae). Journal of Economic Entomology 86: 1714–1719.

Heard, T. A. (1988a). Establishment and propagation of hives of the stingless bee Trigona *carbonaria*. Bee Keeping in the Year 2000. Proceedings of the Second Australian and International Beekeeping Congress, Surfers Paradise, Gold Coast, Queensland, Australia, July 21-26, 1988. Pp 114–116.

Heard, T. A. (1988b). Propagation of hives of Trigona *carbonaria* Smith (Hymenoptera: Apidae). Journal of Australian Entomological Society 27: 303-304.

Heard, T. A (1994) Behaviour and pollinator efficiency of stingless bees and honey bees on macadamia flowers. Journal of Apicultural Research 33: 191–198.

Heard, T. A. (1999) The role of stingless bees in crop pollination. Annual Review of Entomology 44: 183-206.

Heard, T. A (2001). Stingless bees and crop pollination. Bee World 82: 6–7.

Heard, T. A. and A. Dollin (1998). Crop pollination with Australian stingless bees. Australian Native Bee Research Centre, North Richmond NSW.

Heard, T. A. and A. Dollin (2000). Stingless beekeeping in Australia: snapshot of an infant industry. Bee World 81(3): 116-125.

Heard, TA and Hendrikz, JK (1993). Factors Influencing Flight Activity of Colonies of the Stingless Bee Trigona *carbonaria* (Hymenoptera, Apidae). Australian Journal of Zoology 41, 343–353.

Hoffmann, D., J.S. Pettis and P. Neumann (2008). Potential host shift of the small hive beetle (Aethina tumida) to bumblebee colonies (Bombus impatiens). Insectes Soc 55:153–162.

Hornschemeyer, T., R.G. Buetel and F. Pasop (2002). Head structures of Priacma serrata Leconte (Coleoptera, Archostemata) inferred from x-ray tomography. Journal of Morphology 252: 298–314.

Imdorf, A. and L. Gerig (2001).Course in Determination of Colony Strength Swiss Bee Research Centre Swiss Federal Dairy Research Institute, Liebefeld, CH-3003 Bern

Johnson, S.N., D.B. Read and P.J. Gregory (2004). Tracking larval insect movement within soil using high resolution X-ray microtomography. Ecological Entomology 29: 117–122.

Kanao T, Okamoto T, Miyachi Y and Nohara N (2003) Parental exposure to low dose x-rays in Drosophila melanogaster induces early emergence in offspring, which can be modulated by transplantation of polar cytoplasm. Mutation Research 527: 1–6.

Katz AJ and Young SSY (1975) Selection for high adult body weight in Drosophila populations with different structures. Genetics 81: 163–175.

Kerr, WE and Lello, E, (1962) Sting glands in stingless bees. Journal of the New York Entomological Society 70, pp. 190–214.

Lea, A.M. (1910) Australian and Tasmanian Coleoptera inhabiting or resorting to the nests of ants, bees and termites. Proc R Soc Victoria [NS] 23: 116-230.

Lea, A.M. (1912) Australian and Tasmanian Coleoptera inhabiting or resorting to the nests of ants, bees and termites. Proc R Soc Victoria [NS] 25 Suppl: 31-78.

Lehmberg, L., K. Dworschak and N. Bluethgen (2008). Defensive behaviour and chemical deterrence against ants in the stingless bee genus Trigona (Apidae, Meliponini). Journal of Apicultural Research 47:17-21.

Lundie AE (1940) The small hive beetle Aethina tumida, Science Bulletin 220, Department of. Agriculture and Forestry, Government Printer, Pretoria.

Mayack, C. and D. Naug (2009). „Energetic stress in the honeybee Apis mellifera from Nosema ceranae infection." Journal of Invertebrate Pathology: 1-4.

Melo, G A R (1999) Phylogenetic relationships and classification of the major lineages of Apoidea (Hymenoptera), with emphasis on the crabronid wasps. Scientific Papers, Natural History Museum of the University of Kansas 14: 1-55.

Melo, G.A.R; and R.B. Goncalves (2005). Higher-level bee classifications (Hymenoptera, Apoidea, Apidae sensu lato). Revista Brasileira de Zoologia 22(1): 153–159.

Michener C.D. (1960) Observations on the behaviour of a burrowing bee (Amegilla) near Brisbane, Queensland (Hymenoptera, Anthophorinae). Queensland Naturalist 16: 63–67.

Michener C.D. (1961) Observations on the nests and behaviour of Trigona in Australia and New Guinea (Hymenoptera: Apidae). American Museum of Novitates 2026 (08-05-1961): 1-45.

Michener, C.D. (1974). The social behaviour of the bees; A comparative study. Harvard University Press, Cambridge, MA, USA. pp. 404.

Michener, C.D. (2000). The bees of the world. The John Hopkins University Press, Baltimore, MD, USA.

Mutsaers, M. (2006). Beekeepers' observations on the small hive beetle (Aethina tumida) and other pests in bee colonies in West and East Africa. In: Proceedings of the 2nd EurBee conference, Prague, Czech Republic. p 44.

Nates, G. and O. Cepeda (1983). Comportamiento defensivo en algunas especies de meliponínos. Boletín del Departamento de la Biologia de la Universidad Nacional de Colombia, Bogotá 1, pp. 65–81.

Neumann, P. and J.D. Ellis (2008). The small hive beetle (Aethina tumida Murray, Coleoptera: nitidulidae): distribution, biology and control of an invasive species. Journal of Apicultural Research 47:181–183.

Neumann, P., C.W.W. Pirk, H.R. Hepburn, A.J. Solbrig, F.L.W. Ratnieks, P.J. Elzen and J.R. Baxter (2001). Social encapsulation of beetle parasites by Cape honeybee colonies (Apis mellifera capensis Esch.). Naturwissenschaften 88:214-216.

Neumann, P. and W. Ritter (2004). A scientific note on the association of Cychramus luteus (Coleoptera: nitidulidae) with honeybee (Apis mellifera) colonies. Apidologie 35: 665-666.

Neumann ,P. and P.J. Elzen (2004). The biology of the small hive beetle (Aethina tumida Murray, Coleoptera: nitidulidae): Gaps in our knowledge of an invasive species, Apidologie 35: 229-247.

Noll, F B (1997) Foraging behaviour on carcasses in the necrophagic bee Trigona hypogea (Hymenoptera: Apidae). Journal of Insect Behaviour 10(3): 463-467.

O'Toole, C. and A. Raw (2004). Bees of the world. London, Cassell Illustrated.

Perna, A., C. Jost, E. Couturier, S. Valverde, S. Douady and G. Theraulaz (2008). The structure of gallery networks in the nests of termite Cubitermes spp. revealed by X-ray tomography. Naturwissenschaften 95(9): 877-884.

Rayment T (1935) A cluster of bees. The Endeavour Press, Sydney.

Rayment T (1947) A critical revision of species in the zonata group of Anthophora by new characters (Part1). Treubia 19: 1–30.

Roig-Alsina, A. and C.D. Michener (1993). Studies of the phylogeny and classification of long-tongued bees (Hymenoptera: Apoidea). University of Kansas Scientific Bulletin 55: 123–173.

Roubik, D.W. (1989). Ecology and natural history of tropical bees. Cambridge University Press, Cambridge, UK.

Schmid-Hempel, P. 1998 Parasites in social insects. Princeton University Press.

Schmolke, M.D. (1974) A study of Aethina tumida: the small hive beetle. Msc thesis, University of Rhodesia, South Africa; 181 pp.

Serrao, J E (2001) A comparative study of the proventricular structure in corbiculate apinae (Hymenoptera, Apidae). Micron 32: 379–385.

Serrao, J E (2005) Proventricular structure in solitary bees (Hymenoptera: Apoidea). Organisms Diversity and Evolution 5: 125–133.

Serrao, J.E. and C. da Cruz-Landim (2000). Ultrastructure of the midgut epithelium of Meliponinae larvae with different developmental stages and diets. Journal of Apicultural Research 39(1-2): 9-17.

Spiewok, S. and P. Neumann (2006a). Cryptic low-level reproduction of small hive beetles in honeybee colonies. Journal of Apicultural Research 45:47-48.

Spiewok, S. and P. Neumann (2006b). Infestation of commercial bumblebee (Bombus impatiens) field colonies by small hive beetles (Aethina tumida). Ecological Entomology 31:623-628.

Spiewok S, J.S. Pettis, M. Duncan, R. Spooner-Hart, D. Westervelt and P. Neumann (2007). Small hive beetle, Aethina tumida, populations I: Infestation levels of honeybee colonies, apiaries and regions. Apidologie 38:595–605.

Stow, A., D. Briscoe, M. Gillings, M. Holley, S. Smith, R. Leys, T. Silberbauer, C. Turnbull and A. Beattie (2007). Antimicrobial defences increase with sociality in bees. biology letters 3(4): 422-424.

Tollner, EW (1991) X-ray computed tomography applications in soil ecology. Agriculture, Ecosystems and Environment 34: 251–260.

Torto, B., R.T. Arbogast, H. Alborn, A. Suazo, D. Van Engelsdorp, D. Boucias, J.H. Tumlinson and P.E.A. Teal (2007). Composition of volatiles from fermenting pollen dough and attractiveness to the small hive beetle Aethina tumida, a parasite of the honeybee Apis mellifera. Apidologie 38(4): 380-389.

Torto, B, A. Suazo, H. Alborn, J.H. Tumlinson and P.E.A. Teal (2005). Response of the small hive beetle (Aethina tumida) to a blend of chemicals identified from honeybee (Apis mellifera) volatiles. Apidologie 36:523-532.

Wille, A (1979) Phylogeny and relationships among the genera and subgenera of the stingless bees (Meliponinae) of the world. Revista de Biologia Tropical 27: 241–277.

APPENDIX 1

A collaborative work between the Swiss Bee Research Institute and the University of Bern has helped develop new methods for assessing population dynamics in managed A. mellifera colonies.

Preliminary Studies for Bee Population Dynamics using Diagnostic Radioentomology

Mark K Greco, Stefanie Ohlerth, Johann Lang, Peter Gallmann

Introduction

The Liebefeld Method is a well established system for determining population dynamics in managed *Apis mellifera* colonies. Researchers can achieve an accuracy of around 90% once properly calibrated. Interest in the use of x-ray computerised tomography for studying insects (Diagnostic Radioentomology) is increasing. We investigated the potential for improving on the accuracy of The Liebefeld Method using Diagnostic Radioentomology (DR) as an alternative method. Measurements of population size and pollen-wax-honey volumes were performed using DR on nine managed colonies of *A. mellifera*.

Materials and Methods

VETSUISSE

Results

Volume rendering and quantifications with BeeView software

Conclusions

Preliminary results show that DR can produce reliable/reproducible image data including accurate volume measurements for behavioural and analytical experimentation, such as The Liebefeld Method. Further development is required to produce an acceptable methodology for entomologists requiring non-invasive experimental techniques.

mark.greco@alp.admin.ch
CH-3003 Bern
www.alp.admin.ch

Universität Bern | Universität Zürich
vetsuisse-fakultät

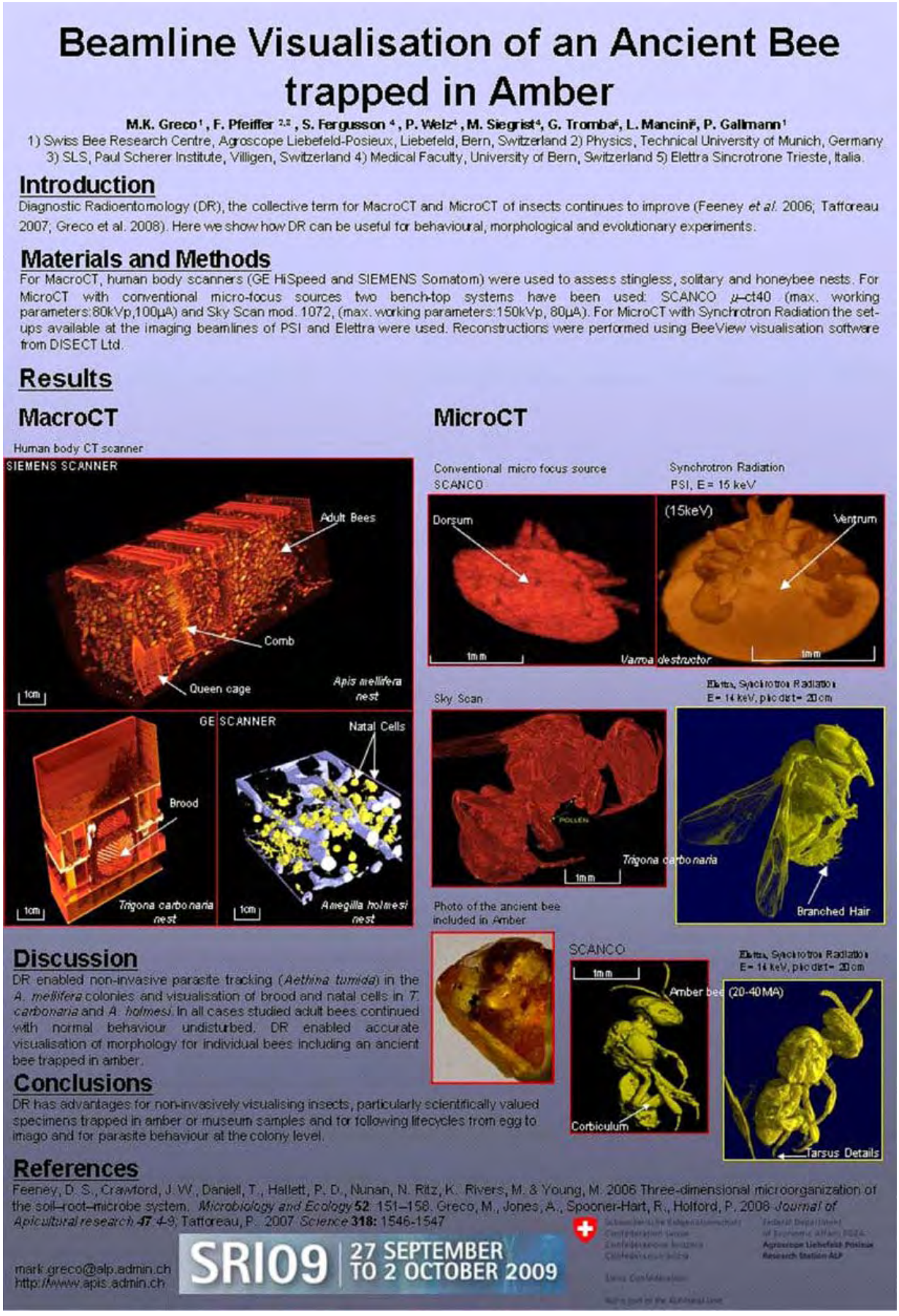
Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Confederation

Federal Department of Economic Affairs DEA
Agroscope Liebefeld-Posieux
Research Station ALP

APPENDIX 2

Details of how DR can be used for non-invasive assessment of individual insects and their colonies.



APPENDIX 3

DR images of a live bee that has been fed a sucrose solution labelled with radiographic contrast. After 1 h, the contrast can be seen in the gut and haemolymph (red, yellow & orange zones arrowed).