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A new technique for monitoring *Trigona carbonaria* nest contents, brood and activity using X-ray computerized tomography

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SUMMARY

Brood status in the Australian stingless bee *Trigona carbonaria* is difficult to monitor non-invasively as splitting the hive damages the nest, and the involucrum prevents observations of internal structures and evidence of queen activity. In addition, increases in hive weight, also used as a measure of colony health, can be attributed to increased pollen and nectar collection by foragers in times of colony stress rather than improved brood status. To assess brood status and queen activity, we performed helical two-dimensional and three-dimensional X-ray computerized tomography (CT) to measure brood chamber volume in a *T. carbonaria* hive. All previously reported nest structures including larval cells, pupal cocoons, involucrum, cerumen and batumen layers were clearly identifiable. In addition, the on-screen linear callipers enabled accurate estimates of brood chamber volume to be made. A batumen bridge was found that may secure the brood chamber to the base of the hive box; this structure has not been reported before. CT could be used to follow the life cycle of stingless bees, track the development of natural nests and observe nest morphology to distinguish between species of *Trigona*. In addition, sequential scans will be useful in non-invasively assessing changes in brood status.

Keywords: *Trigona carbonaria*, stingless bees, nests, hives, brood, X-ray computerized tomography

INTRODUCTION

The Australian stingless bee, *Trigona carbonaria* (Apidae: Meliponini), is a small- to medium-sized bee which has a vestigial sting (Dollin *et al*., 1997). It is found in the tropical north of Australia and along the eastern coast as far south as Bega (36° 40' S, 149° 49' E), New South Wales (Dollin, 1996). It is considered to have a primitive form of eusociality. Colonies have male drones and two female castes – the queen and sterile workers (Dollin *et al*., 1997). Bees of this species are less harmful to humans and domesticated and native animals than the introduced European honey bee, *Apis mellifera*, because they are stingless (Heard, 1999).

Trigona spp. are important pollinators of Australian native plants (Dollin, 1996). However, their potential value in crop pollination and honey production has not been investigated widely (Heard, 1999), although they are used to a limited extent in commercial crop pollination, particularly macadamias (*Macadamia integrifolia*) (Heard, 1999). The agricultural importance of *T. carbonaria* is likely to increase in the future in the event of reduced pollination effectiveness of *A. mellifera* due to the introduction to Australia of Africanized strains as well as bee diseases and parasites. *A. mellifera* also has an inherent low efficiency of pollination in some crop species and may have climatic limitations in areas where *T. carbonaria* thrive (Heard, 1999).

To manage *T. carbonaria* effectively, it is essential to monitor colony health. Traditionally, colony health 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. Colony health can also be assessed by manually splitting the hive box apart to view internal structures and evidence of queen activity (Dollin & Heard, 1997). However, the involucrum, a reticular structure made from cerumen that structurally supports the brood and aids in nest ventilation, prevents detailed observations of internal nest structures and 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. However, bees quickly smear this with cerumen, which is a mixture of their wax and resinous propolis (Amano *et al*., 2000) or batumen which is made from cerumen and mud (Amano *et al*., 2000). This excludes light from the hive contents (Dollin *et al*., 2001) and prevents adequate visualization of nest structures and queen activity.

X-ray computerized tomography (CT) has been used as a non-invasive method to visualize internal human morphology since the early 1970s and, more recently, has been used to study soil ecology and insect movement (Tollner, 1991; Harrison *et al*., 1993; Fuchs *et al*., 2004; Johnson *et al*., 2004). In this paper we detail the use of CT for non-invasively observing *T. carbonaria* nest structures within a manufactured wooden hive box. We used helical, low dosage, two- and three-dimensional scans to view and measure the involucrum, cerumen coverage, batumen, pollen stores, honey stores and brood content and volume.

MATERIALS AND METHODS

A hive box (fig. 1) designed by Heard (1988) containing a healthy, actively growing colony of *T. carbonaria* was used for CT. 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 placed on this sheet with the entrance

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TABLE 1. Parameters for CT used for imaging the *Trigona 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 × 512	512 × 512
Voxel size	0.449 mm^3	0.449 mm^3

towards the foot of the bed. 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 nest structures were accurately localized for scanning by utilizing a lateral 'scout view' (fig. 2); such views allow the CT operator to plan a scan sequence according to the structures visualized. The brood was measured in vertical, horizontal and orthogonal planes using on-screen linear callipers. The

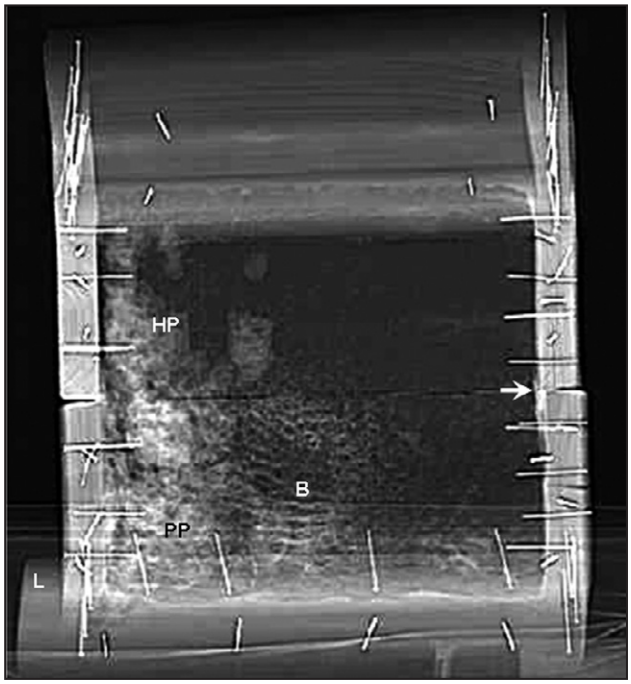

FIG. 2. Two-dimensional image of the hive taken in the vertical plane showing the landing board (L), honey (HP) and pollen (PP) pots, the brood chamber (B) and batumen (arrowed).

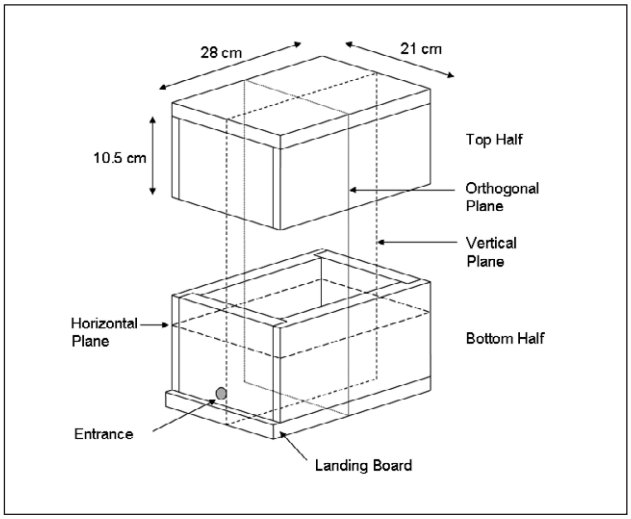

FIG. 1. Diagram of the hive box designed by Dr Tim Heard (reprinted with permission from the Australian Native Bee Research Centre) for *Trigona carbonaria* and showing planes of scanning.

volume of the brood chamber was then estimated using the formula $4/3\pi r_1.r_2.r_3$ where r_1, r_2 and r_3 are the radii of the chamber in the three planes scanned and assuming that the brood chamber was approximately ellipsoid. Further evaluation of the nest structures was performed using virtual imaging software (eFILM-LITE v. 1.5.0.0-DICOM, Digital Imaging and Communications in Medicine NEMA) in cine mode. After scanning, the hive was physically split and viewed optically to confirm the CT interpretations. Separate scans of pollen, honey and batumen were also performed to determine their level of X-ray attenuation (CT number) for direct comparison with the honey, pollen and batumen visualized within the hive.

RESULTS

Two-dimensional scans of the hive were made and nest structures are shown in the vertical (fig. 2) and orthogonal plane (fig. 3). These scans enabled accurate localization of all previously reported internal nest structures (Amano *et al.*, 2000) and, under the high resolution settings, the hive received a radiation dose

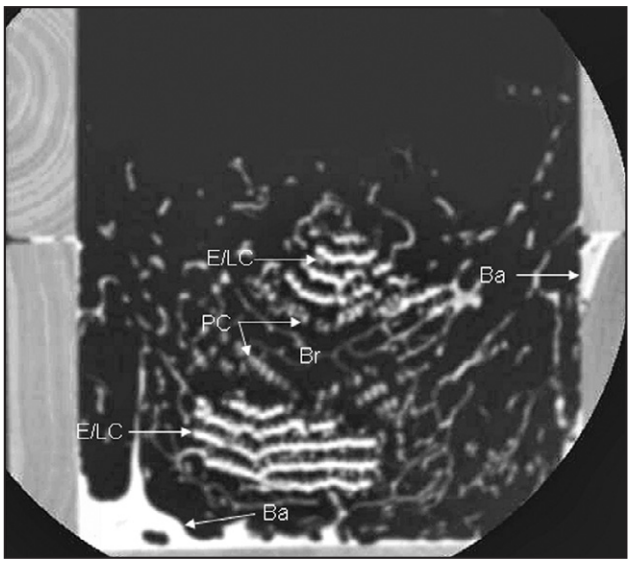

FIG. 3. Two-dimensional image taken in the orthogonal plane of the brood chamber (Br) showing egg or larval cells (E/LC), pupal cocoons (PC) and batumen (Ba).

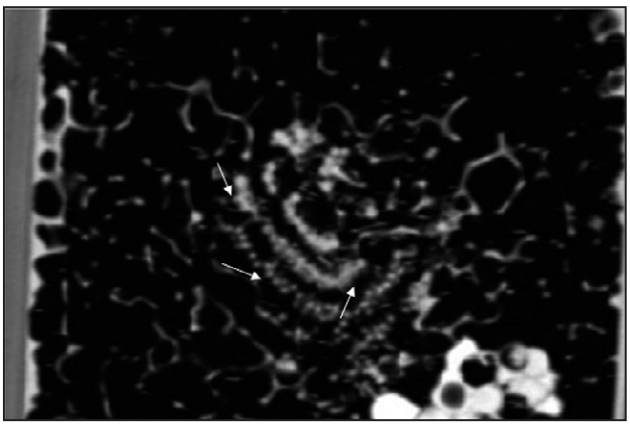

FIG. 4. Two-dimensional image of the brood showing spiral structure (arrowed), a feature that is characteristic of *Trigona carbonaria*.

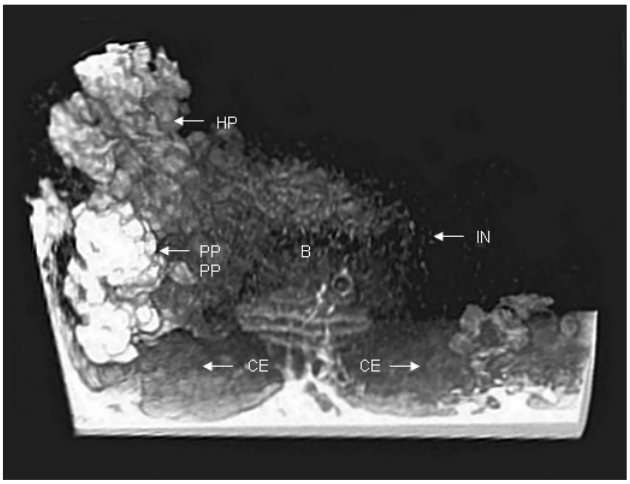

FIG. 5. Three-dimensional reconstructed image detailing honey (HP) and pollen (PP) pots, brood chamber (B), cerumen (CE) and involucrum (IN).

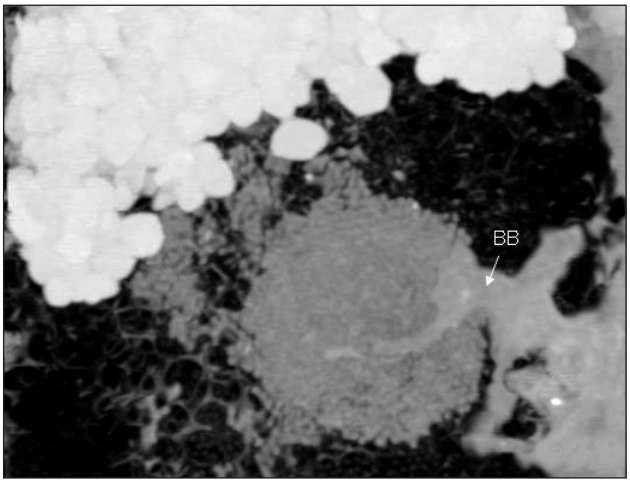

FIG. 6. Three-dimensional view of the batumen bridge (BB) extending into the brood chamber.

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 samples of honey (+319) and pollen (–376). 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 interface between the top and bottom boxes, joints and cracks within the wood structure.

The 2-D 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 1170 ml in volume. Within the brood chamber, egg and larval cells could be distinguished from pupal cocoons (fig. 3). However, egg and larval cells could not be distinguished from each other. In this hive, the brood consisted of a spiral shaped structure (figs 3 and 4) in which individuals within the brood progressed through developmental stages. The stage of the brood development progressed from the apex to the base of the structure with rows of cells containing eggs or larvae being visualized at the apex and pupal cocoons at the base. Two groups of egg or larval cells were present, the top rows of which were 29 mm and 83 mm, respectively, from the bottom of the hive box separated by a large group of pupal cocoons.

Computer-generated, three-dimensional images are shown in figures 5 and 6. These images give a clear representation of the positional relationships between the hive structures. In fig. 5, the spherical nature of the pollen and honey pots is clearly evident as is the grouping of most pollen pots together closer to the entrance of the hive and the grouping of the honey pots above them and further to the centre of the nest. 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 larval cells and the upper layers of pupal cocoons that appears less dense. Figure 5 also shows the relationship between the brood chamber and the other structures of the nest; in this case the brood constitutes approximately 1/3 of the occupied region of the hive box.

Figure 6 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 into the centre of the brood chamber.

DISCUSSION

X-ray computerized tomography allowed the internal structures of the nest to be viewed and assessed non-invasively. This new approach is non-destructive to the nest and bees, in contrast to the traditional method of splitting the hive apart to view internal structures. In particular, because of the ability of CT to accurately localize and reproduce structures spatially, we were able to calculate brood chamber volume and structural components such as egg or larval cells and pupal cocoons. Future scans of progressive temporal stages may enable the differentiation of egg from larval cells. The accurate measurement of brood volume enables assessment of queen activity and, therefore, colony health. Also, through a series of scans, it would be possible to monitor temporal changes in brood chamber volume to assess changes in colony health more accurately than the current method of using hive weight, with negligible disruption or damage to wild or managed stingless bee nests. Whilst we view this technique mainly for research purposes, colonies in logs or hive boxes could be transported easily to CT facilities enabling the use of this technique by beekeepers.

The total dosage of radiation for the scan was 13.9 mGy which was spread over 477 tomographic slices, each 1-mm thick. 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 *c*. 3800-times less than the minimal dose required for a biological effect to occur in *Drosophila melanogaster* (Kanao *et al*., 2003). *D. melanogaster* are less than half the size of *T. carbonaria*; therefore, the radiation dose absorbed by each unit mass of bee is less, and this

reduces the risk of a biological effect on scanned bees even further. Whilst we found it prudent to limit the radiation dosage to only one scan of the hive for this study, the resultant dosage demonstrated that this technique can be used repeatedly on future study hives with no detectable biological effects on the colony.

The batumen bridge found in this study has not been previously described. It may be an anomaly for this particular nest but could also be a normal structure in *T. carbonaria* nests that secures the brood chamber to the base of the hive box. Further scans of a series of nests 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, CT could be used to follow the life cycle of stingless bees from egg to imago and may be useful for determining interactions between development and environmental factors (A Dollin, personal communication). Whilst CT 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 CT 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, CT 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 programmes.

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