



**Investigations on the behaviour of the Australian native bee,  
*Austroplebeia australis*, with particular reference to its defence  
strategies against the African small hive beetle, *Aethina tumida*.**

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## **Declaration**

I certify that, to the best of my knowledge, this thesis does not contain any material that has been accepted for the award of a degree or diploma. Neither does it contain any material that has been previously published or written by another person, except when due reference has been made in the text of this thesis. Unless otherwise indicated, all data and observations presented herein are the results of my own work. This thesis does not contain material that is subject to copyright restrictions.

A handwritten signature in black ink, appearing to be 'Megan Halcroft', written in a cursive style.

Megan Halcroft

April 2007

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## **List of Abbreviations**

SHB: small hive beetle

OP: observation platform

CT: controlled temperature

UWS: University of Western Sydney

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# 1            Summary

The African small hive beetle (SHB), *Aethina tumida*, is a newly (2001) introduced honeybee parasite and is a potential threat to the sustainability of the honeybee (*Apis mellifera*) and, therefore, insect-pollinated horticultural and agricultural crops. The Australian native stingless bee, *Austroplebeia australis*, builds colonies based around honey, pollen, wax and brood, all of which are the preferred food source of SHB. Little is known about in-hive behaviour of *A. australis*, particularly its defensive behaviour.

To investigate behaviour of *A. australis* and its vulnerability to SHB invasion, a non- disruptive method of hive observation was applied to four bee colonies. An observation platform (OP) was designed and incorporated into *A. australis* hives, to enable the observation and assessment of the normal hive behaviour of the bees. The newly propagated colonies were maintained in controlled temperature (CT) rooms and provided with supplementary food reserves to build up hive strength before the introduction of different life stages of the SHB. These studies conducted prior to SHB introduction confirmed the view that these tropical stingless bee colonies could be maintained and strengthened under controlled conditions and that their behaviour could be observed, without disruption, with the aid of an OP nest extension. The OPs allowed observation of previously-reported normal hive behaviour, confirming their usefulness for future studies. They also enabled recording of previously unreported behaviour

All life stages of SHB were then introduced to the colonies and the bees' behaviour toward hive invasion was assessed. Following introduction, 96% of the SHB eggs were consumed or destroyed within a 90 minute period. All introduced eggs were consumed or destroyed within 24 hours. Three-day old SHB larvae were introduced into each hive OP and removal by the bees was observed over a 15 minute period. There was significantly ( $p < 0.001$ ) increased efficiency in larval removal by the bees between the first time larvae were introduced into the OP, compared with the second time they were introduced, and all larvae were removed after two hours. SHB adults were also introduced directly into OPs, to simulate invasion under circumstances of hive damage or splitting, and also via the hive entrance, to assess the ability of *A. australis* to defend an undisturbed hive. Directly introduced SHB adults were removed or totally incapacitated by entombment in resin within six hours of introduction.

Initially, SHB adults introduced via the hive entrance penetrated the defences of *A. australis* guards but data analysis shows that there were significantly more adults ejected in the third attempt than in the first attempt. Ejection rates of SHB adults by guard bees increased

significantly ( $p = 0.017$ ) between the first and third introductions. All SHB that infiltrated the hive were removed or destroyed within 60 hours of the last beetle introduction.

It was concluded that strong, healthy *A. australis* colonies, maintained in undamaged hives are able to defend themselves against invasion by SHB.

## 2 Literature review

### 2.1 The Small Hive Beetle

#### 2.1.1 Introduction to the small hive beetle

The African small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae), was first identified by Murray (1867) when he received two specimens from Rev. W.C. Thomson from Old Calabar in southeastern Nigeria, West Africa. The insect was not referred to again until Lundie conducted extensive studies on the beetle in 1940 (Taber 1999). Further studies were carried out by Lundie (1951; 1952) and by Schmolke in (1974). Both authors concluded that this beetle, which is native of sub-Saharan Africa (Hepburn and Radloff 1998) was a parasite and scavenger of African honeybee subspecies (*Apis mellifera scutellata*) and was of no significant economic threat in South Africa. However, some damage may occur in central Africa (Mutsaers 2006), for example during the rainy season (Mutsaers 1991). While this may be true in its native environment, the small hive beetle (SHB) has, in more recent years, shown itself to be of considerable threat to European honeybee subspecies (Elzen *et al.* 1999; Hood 2000; Taber and Hood 2000; Mostafa and Williams 2002; Ellis *et al.* 2003d; Gillespie *et al.* 2003; Neumann and Elzen 2004).

#### 2.1.2 The small hive beetle in its native range

In its native range, the SHB coexists with African honeybee, causing little or no damage in host colonies, unless the colony is weak, diseased or has abandoned its hive (Hepburn and Radloff 1998; Taber 1999; Elzen *et al.* 2001; Ellis *et al.* 2003d; Ellis *et al.* 2004c; Neumann and Elzen 2004; Ellis and Hepburn 2006).

The main problem encountered with the SHB in its natural habitat is the destruction of honey products, usually within the honey house (Wenning 2001; Mostafa and Williams 2002; Neumann and Elzen 2004). SHB are highly attracted to bee products, that of pollen and honey, especially in combination with adult bees (Elzen *et al.* 2000a; Suazo *et al.* 2003; Torto *et al.* 2005). If bee products are left unguarded, the SHB can destroy a honey super within two weeks (Wenning 2001).

It has been found that African honeybee colonies which are heavily infested with adult beetles can remain in good health (Neumann and Elzen 2004). The bees are able to confine the SHB by harassing the adults and constructing prisons made from propolis, produced from a mixture of wax and tree resin (Neumann *et al.* 2001a; Ellis *et al.* 2004c; Ellis *et al.* 2004b). The SHB has

even developed the ability to elicit food from its host (Ellis 2002b). It mimics a hungry bee by tapping on the head of the guard bee with its antennae which induces the guard to regurgitate honey through trophallaxis (Ellis 2002b). The SHB is not always successful in its efforts but may be so often enough to maintain a food supply within its prison confines (Ellis and Hepburn 2006).

African honeybees are very efficient at removing SHB eggs and larvae from the hive (Neumann and Elzen 2004). The bees have a remarkable ability to detect hidden eggs within sealed brood cells and they abort the brood as well as remove the beetle eggs (Ellis *et al.* 2003e). Some honeybees have been observed eating SHB eggs within the hive (Spiewok and Neumann



**Figure 1: African honeybee jettisoning SHB larva (Neumann and Härtel 2004).**

2006a). If larvae are found in the hive, the bees remove them by “jettisoning” them, up to 20 meters from the entrance (Figure 1) (Neumann and Elzen 2004; Neumann and Härtel 2004). It is not until the African honeybee, which has a habit of swarming non-reproductively (Hepburn and Radloff 1998), absconds from the nest and leaves behind bee products such as honey, pollen and brood that the SHB is able to freely

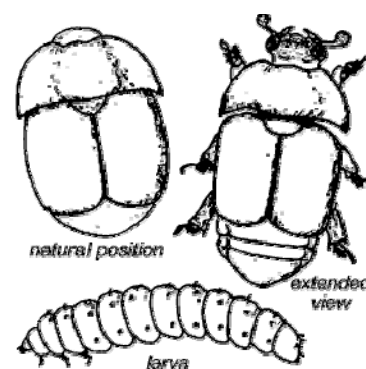
lay masses of eggs without interference (Taber 1999; Neumann and Elzen 2004).

### 2.1.3 The biology and life cycle of the small hive beetle

The adult African small hive beetle is dark brown to almost black in colour (yellowish-brown after eclosure) and measures between 5-7mm in length. It is an oval shape and has clubbed antennae. Its exoskeleton is extremely tough and sclerotized making it difficult for the bees to grasp (Ellis and Hepburn 2006). This exoskeleton also protects the beetle from the bees’ stings. When

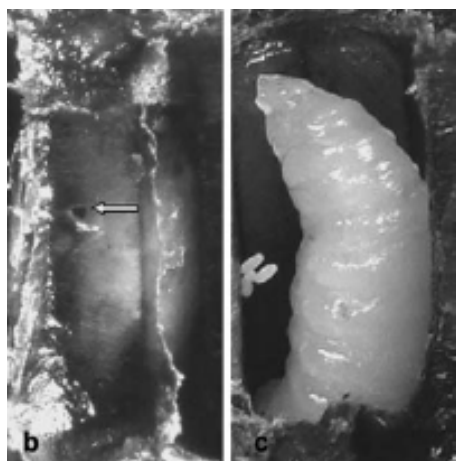
confronted by a bee, the beetle simply stops still, retracts its legs and head and “turtles” under its shield (Figure 2)

(Neumann *et al.* 2001a; Neumann and Elzen 2004; Ellis and Hepburn 2006).



**Figure 2: Small hive beetle adult and larva. Note the protective “natural” position. (Sanford 1999)**

One female SHB is capable of laying at least 13 eggs each day, equating to an average of 1000 eggs in three months (Mostafa and Williams 2002). She lays her pearly white, 1.4mm x 0.26mm eggs in irregular clusters in crevices within the hive, into empty cells or into open brood cells containing bee eggs or larvae (Ellis *et al.* 2003e; Neumann and Elzen 2004; Neumann and Härtel 2004). The female SHB can also oviposit directly into sealed brood, containing pupa, by puncturing a small hole in the brood capping (Ellis *et al.* 2003a) or through the mid rib of the comb and ovipositing into the adjoining cell (Figure 3) (Ellis *et al.* 2003e).



**Figure 3:** (b) shows the hole where the ovipositor punctured the midrib of the cell (c) shows a small cluster of SHB eggs inside the brood cell (Ellis *et al.* 2003e)

The larvae hatch in 1-6 days, with most hatching in the first 2-3 days (Lundie 1940; Neumann *et al.* 2001a).

Larvae are approximately 5mm in length and at maturity will have grown to 10-11mm. Time from hatching to larval maturity averages 10-16 days. During the larval stage SHB crawl around the hive comb, burrowing through the comb, eating debris, honey, pollen and preferentially bee brood (Lundie 1940; Neumann and

Elzen 2004; Spiewok and Neumann 2006a). The larvae defaecate throughout the hive which causes the honey to become discoloured, fermented, frothy and foul smelling

(Taber 1999; Wenning 2001; Mostafa and Williams 2002; Ellis *et al.* 2003a; Neumann and Elzen 2004; Ellis and Hepburn 2006). The condition of the hive deteriorates to the point where the honey drips from the cells, the wax melts and the entire hive contents ooze from the hive entrance (Wenning 2001; Neumann and Elzen 2004; Ellis and Hepburn 2006). SHB are able to form swarms and enter a bee colony *en masse* (Tribe 2000) and although African honeybees may tolerate high numbers of SHB adults within the colony (Neumann and Elzen 2004), if the level of the beetle population increases rapidly, there may be a point where the colony is forced to abscond. This leaves the hive products and brood unprotected and available for consumption by the SHB (Ellis *et al.* 2003d; Neumann and Elzen 2004) while reproduction goes unchecked.

When fully mature, the larvae reach the “wandering phase”, become positively phototactic and leave the hive to pupate in the soil (Lundie 1940; Schmolke 1974; Neumann and Elzen 2004). They burrow into the soil, up to 20 cm deep but usually within the first 10 cm (Pettis and Shimanuki 2000). The pupae can be found as far as 30 m away from the hive entrance, as they searched for suitable soil in which to pupate (P. Neumann, pers. comm. March 2007). When suitable soil can be found in close proximity to the hive, most larvae burrow within a 30 cm

radius and none further way than 2 m (Pettis and Shimanuki 2000). Ellis et al (2003d) found the ideal soil type for burrowing and pupation is moist and sandy. No reports of serious beetle infestation in the heavy clay soil area of Georgia, in the USA, were recorded (Wenning 2001). Hot, dry conditions are not conducive to beetle reproduction and may limit its spread to some extent, especially in drought prone areas (Neumann and Elzen 2004). The time spent in the soil may vary between 7 days and up to three months (JS Pettis & P. Neumann, unpublished data) depending on moisture and temperature. Wenning (2001) believes adult SHB may be attracted to stressed hives up to 16 km from the original contamination source. Schmolke's studies (1974) showed that beetles did not fly before dusk but Elzen et al (2000b) recorded significant beetle flight activity between 16:00 hr and complete sunset (21:00 hr).

#### 2.1.4 Male / female variability in small hive beetle

The female adult is able to oviposit within one week of emerging from the soil (Lundie 1940). Studies have shown the production of a higher female to male ratio on most diets (Ellis *et al.* 2002b), and these higher female ratios have also been seen in natural populations (Ellis *et al.* 2002a). Female larvae are usually larger and heavier than males (Lundie 1940), providing them with more stores to survive burrowing processes and pupation (Ellis *et al.* 2002a; Ellis *et al.* 2002b; Ellis *et al.* 2004d). Higher weights may be due to increased food consumption during larval development (Ellis *et al.* 2002a).

#### 2.1.5 Nutritional requirements of the small hive beetle

The adult beetle is most attracted to the contents of a “living” hive, whereas pollen and honey alone are less attractive (Elzen *et al.* 1999; Ellis *et al.* 2002b; Elzen *et al.* 2002; Neumann and Elzen 2004). They can, however, survive on a range of food sources including fresh and rotten fruit, pollen, honey, brood comb, bee brood and bee eggs (Ellis *et al.* 2002b). Reproduction is not possible on empty brood comb or honey alone (Ellis *et al.* 2002b). Adults have been found to survive for up to nine days without food or water (Elzen *et al.* 2000b; Flügge 2001; Ellis 2002a; Ellis *et al.* 2002b). The highest rates of reproduction have been seen with adults and larvae fed on a combination of honeybee colony products, such as pollen, honey and bee brood (Elzen *et al.* 2000b; Neumann *et al.* 2001b; Ellis 2002a; Ellis *et al.* 2002b; Mürrle and Neumann 2004; Neumann and Elzen 2004).

Mass rearing of SHB is utilized for scientific observation and experimentation on the pest and its host. Mass rearing may be accomplished by placing adult beetles, together with pollen,

honey and brood containing comb (Neumann *et al.* 2001b; Mürle and Neumann 2004) into rearing boxes. Mürle and Neumann (2004) reared 36,000 adult SHB in 63 days from just 80 parents. This gives an indication of the fecundity of this pest, given the right environment. Haque and Levot (2005) devised a rearing method that does not require honeybee comb or brood. This enabled rearing of the beetles under more controlled, laboratory conditions. A moist environment was maintained at all stages of the rearing. To simply maintain the adult beetles, sugar crystals were provided as the only food source. In order to generate reproduction protein sources were provided in the form of pollen and yeast. An oviposition media was included in the rearing box. In all techniques of mass rearing it is necessary to provide a moist soil media for pupation (Mürle and Neumann 2004; Haque and Levot 2005). In its native environment, the SHB is capable of producing five generations in a single year (Lundie 1940), but this may be lower in more temperate climates (Neumann *et al.* 2001b; Neumann and Elzen 2004) Adults can live for up to six months or even longer (Ellis *et al.* 2002b).

### 2.1.6 Effects of the small hive beetle on hosts outside its native range

Outside its native range, the SHB has shown itself to be capable of total hive destruction and has wreaked havoc in apiaries throughout the United States (Elzen *et al.* 1999; Hood 2000; Taber and Hood 2000; Ellis *et al.* 2003a; Neumann and Elzen 2004), Australia (Fletcher and Cook 2002; Gillespie *et al.* 2003) and Egypt (Mostafa and Williams 2002). The speed with which the SHB has spread in the last ten years has led scientists and beekeepers alike to review the beekeeping methods that have been in place for decades (Wenning 2001; Mostafa and Williams 2002; Hood and Miller 2005).

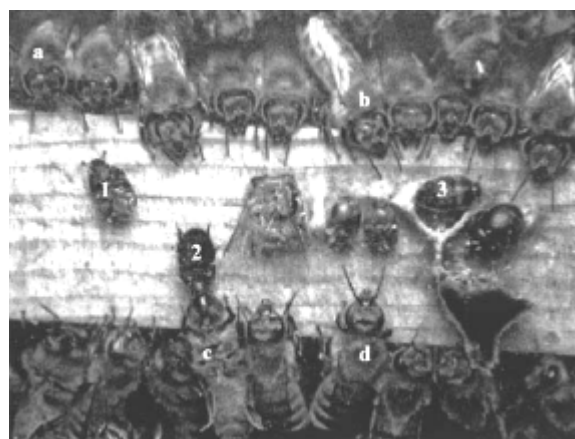
Unlike the African honeybee, European honeybees appear to be less aggressive toward the SHB invaders resulting in higher levels of beetle reproduction within the hive (Elzen *et al.* 2000a; Hood 2000; Taber and Hood 2000; Neumann *et al.* 2001a; Wenning 2001; Ellis 2002b; Mostafa and Williams 2002; Ellis *et al.* 2003b; Ellis *et al.* 2003c; Ellis *et al.* 2003e; Ellis *et al.* 2004a; Ellis *et al.* 2004c; Ellis *et al.* 2004b; Neumann and Elzen 2004; Neumann and Härtel 2004; Ellis 2005). Detection of SHB eggs within brood cells is also less efficient in the European subspecies (Ellis *et al.* 2003a; Ellis *et al.* 2003e). These traits, combined with undesirable beekeeping practices, may result in a high infestation level of adult beetles and high rates of reproduction within honeybee colonies. If left unchecked, massive numbers of larvae can decimate an entire colony in a matter of days or weeks (Wenning 2001). The resulting

destruction causes the unprepared colony to abscond (Ellis *et al.* 2003a; Neumann and Elzen 2004). This may lead to colony death if it has insufficient time to locate and build up a new hive before winter.

### 2.1.7 Confinement of small hive beetle by honeybee species

Both European and African honeybees construct propolis prisons (Figure 4) within the hive peripheries to control, to some extent, the movement and reproductive behaviour of adult SHB (Neumann *et al.* 2001a; Ellis *et al.* 2003c).

European bees are able to confine the beetles to their prisons while SHB populations are low, but it has been speculated that when beetle numbers are high, European bees can no longer confine the beetles, enabling them to roam freely, mate and reproduce (Elzen *et al.* 2001; Ellis 2002b; Ellis *et al.* 2003c).



**Figure 4: Prison constructions and confinement of SHB by honeybees. (Ellis *et al.* 2003c)**

### 2.1.8 Adaptive behaviour of the small hive beetle

Confined SHB adults have survived for over two months in hive prisons, by eliciting food from their prison guards through mimicry of antennal contact, and stimulation of trophallactic behaviour, (Elzen *et al.* 2001; Neumann *et al.* 2001a; Ellis *et al.* 2002c; Ellis 2005). SHB adults are able to survive cold climates by overwintering inside honeybee clusters (Neumann and Elzen 2004). Pettis and Shimanuki (2000) found up to 300 SHB inside European bee clusters, where thermoregulation is used to maintain hive warmth during winter. Once the cold period has passed the adult beetles are able to reproduce quickly (Ellis *et al.* 2002b). SHB have also been seen flying with absconding swarms of bees (Ellis *et al.* 2003a), guaranteeing them a suitable habitat and an appropriate food source.

### 2.1.9 The spread of the small hive beetle pest and its economic impact

It is still unclear how the SHB was first transported out of its native range and into areas where European honeybees are farmed (Neumann and Elzen 2004). It is thought that their ability to survive on a diet of fruit may have led to SHB transport by ship from Africa to the ports at Florida, South Carolina and Georgia in the USA (Hood 2000).



**Figure 6: Shaded areas indicate countries affected by SHB in European bee hive**



**Figure 5: Shaded states indicate parts of USA affected by SHB up to 2004**

Unidentified specimens of adult SHB were recorded in South Carolina as early as 1996 and official reports came from Florida in 1998 (Neumann and Elzen 2004). Further spread of the pest has been through the migratory habits of honey producers in their efforts to follow nectar flows (Delaplane 1998; Wenning 2001; Neumann and Elzen 2004).

In 1998 the Florida honey industry lost an estimated \$3 million through hive destruction (Ellis *et al.* 2002b) and by 1999 SHB had spread to 12 states. By 2000 it had spread across 15 states and in March 2003, 29 states reported the presence of SHB (Neumann and

Elzen 2004). Hood (2004b) shows those Figures have further increased to 32 states in the US alone (Figure 5). In 2002 SHB was discovered in Manitoba, Canada. After extensive studies in 2003 there was no SHB detected in local apiaries (Hood 2004) but it has recently been reintroduced into Canada (CHC 2006). Investigations showed that the first incidence resulted from importation of contaminated wax products from Texas (Hood 2004) but the second, more recent, contamination came from packaged bees imported from Australia. Neither incidence has resulted in hive damage.

SHB were known to inhabit honeybee colonies throughout sub Saharan Africa and is considered a major pest to European beekeepers in Uganda and Nigeria. It was first reported as far north as Egypt in June 2000 (Figure 6) (Mostafa and Williams 2002).

SHB were found in hives in Richmond, NSW, Australia as early as 2001 (M. Duncan pers. comm. 2003) and were officially recorded in July 2002 (Neumann and Elzen 2004). By 2003

the SHB had moved north, reaching into Queensland and west to Cowra and Binalong (Gillespie *et al.* 2003). In 2005 SHB presence was reported in north-west Victoria and the Goulburn Valley (Goodman *et al.* 2005) and it had progressed north to Townsville by 2006 (Duncan M. pers. comm. October 2006).

### 2.1.10 Methods of controlling small hive beetle

After extensive studies the main recommendation for the control of SHB is improved hygienic practices in and around the apiaries and maintenance of strong hives (Baxter *et al.* 1999; Hood 2000; Wenning 2001; Mostafa and Williams 2002). Strong honeybee colonies can better guard honey, pollen or brood-filled comb because bee numbers are high. Weak hives should be removed, or combined to improve their strength (Hepburn and Radloff 1998; Wenning 2001; Hood 2004; Neumann and Elzen 2004).

Removal of alternative food sources in and around orchards is recommended. Fresh fruit within orchards or fields should be monitored (Eischen *et al.* 1999; Ellis 2002a; Ellis *et al.* 2002b; Mostafa and Williams 2002).

Soil treatments for larval infestation is highly recommended (Hood 2000; Mostafa and Williams 2002; Hood 2004) to break the SHB life cycle (Wenning 2001; Ellis *et al.* 2004d). Treatments include drenching with insecticides (Hood 2000; Wenning 2001; Mostafa and Williams 2002; Hood 2004) or a salt solution (Mostafa and Williams 2002). In-hive treatments with miticides have been successful when used in conjunction with coumaphos (Hood 2000; Hood and Miller 2003). In-hive treatments are not recommended when temperatures are low and beetle activity is reduced (Hood 2000). Soil drenching is the only synthetic chemical treatment utilised in Australia (Anon. 2006) as there is no in-hive treatment currently used (pers. com. M. Duncan, March 2007).

Hygienic practices within the honey house can reduce cross contamination. Extracted comb can be cleaned with household bleach (Park *et al.* 2002). Walls and floors can be hosed down with hot water (Wenning 2001). A night light at floor level can be used to attract mature larvae and the accumulated larvae can be destroyed the next morning (Wenning 2001).

Dead or extremely weak bee hives should be frozen to kill all life stages of the SHB (Mostafa and Williams 2002; Hood 2004). Various “harmless” products such as alcohol, beer, honey, cider vinegar and mineral oil were trialed by Hood and Miller (2003) but the trap design was inefficient, especially for large scale honey producers (Hood 2004).

### 2.1.11 Possible alternative bee hosts

In laboratory trials SHB were able to complete an entire life cycle when introduced into bumble bee (*Bombus impatiens*) colonies (Ambrose *et al.* 2000; Stanghellini *et al.* 2000) and field trials carried out by Spiewok and Neumann reinforced concerns regarding alternative hosts (2006b). The American bumble bee naturally lives in hives below the ground, providing soil in which the SHB can pupate and complete their life cycle (Neumann and Elzen 2004).

It has been hypothesized that the SHB may find alternative hosts in its new range and thus the potential for native bee population invasion is considerable (Neumann and Elzen 2004).

Australia is home to an extensive range of native bee species, several of which store and produce the SHB's favoured food source, namely honey, pollen, wax and bee brood and therefore provide high potential as new hosts (Neumann and Elzen 2004). Experimental work carried out in December 2005 at the University of Western Sydney, Hawkesbury, (pers. comm. P. Neumann, M. Duncan, R. Spooner-Hart, A. Dollin and D. Hoffman 2005) suggests that the Australian stingless bee, *Trigona carbonaria*, is able to fight off SHB intruders by entombing and incapacitating them using resinous substances that are stored within the nest. This appears to be the only investigation conducted to test this hypothesis.

Several authors (in particular Neumann *et al.* 2006) have raised concerns about the possibility of SHB switching its host preference in new geographical locations especially in the absence of its original sympatric host, the African honeybee. Outside its native range the SHB is capable of inducing total colony collapse within *A. mellifera* species. SHB not only invades managed honeybee colonies; it can also invade and destroy feral colonies. If the SHB facilitates the destruction of *Apis* colonies, including feral ones, it will, in turn, destroy its primary host. While the removal of feral honeybee competitors may be considered an advantage (Paini 2004) for populations of native bees, the removal of the primary host of the SHB may result in a host switch which may, subsequently, pose a serious threat to the native bee populations within its new range (Neumann *et al.* 2006).

## 2.2 *Austroplebeia australis*

### 2.2.1 Introduction to the stingless bee, *Austroplebeia australis*

Bees are so classified because of their ability to gather pollen and nectar, on which they raise their young (Michener 2000). Australian stingless bees are highly eusocial insects, within the family Apidae and the sub-family Meliponinae (Rayment 1935; Michener 1974). There are only

two genera of stingless bees in Australia, *Trigona* and *Austroplebeia* (Dollin 1997). Their distribution is limited by climate and they thrive in tropical Australian regions from Cape York in northern Queensland to central New South Wales. *Trigona* species can be found as far south as Dungog in NSW, (35°S) (Figure 7) (Dollin *et al.* 1997; Michener 2000), but this climate is marginal for their survival. *Austroplebeia australis* inhabits the tropical regions of Western Australia, the Northern Territory, Queensland and northern New South Wales. It was originally described as *Trigona australis* but was reclassified by Michener (1990) as *Austroplebeia australis* (Dollin 1996c; Dollin 1997).

Australian stingless bees nest in natural and manmade cavities such as trees and logs, rock crevices, within termite mounds, in fence posts, door hollows and coconut husks (Dollin 1996c). *Trigona mellipes*, in the Northern territory, can be found underground in old termite mounds (pers. comm. Russell Zabel March 2007). *T. carbonaria*, *T. hockingsi* and *A. australis* can be maintained in man-made OATH (Original Australian Trigona Hive) boxes and farmed for their honey and resinous by-products or as crop pollinators (Dollin and Heard 1999; Dollin *et al.* 2000; Dollin *et al.* 2001).



**Figure 7: Distribution of Australian stingless bees. (Dollin and Dollin 2002)**

Stingless bees perform a pollination service whilst foraging amongst a wide variety of native and exotic flowers (Michener 1974; Sommeijer *et al.* 1983; Wille 1983; Adams and Lawson 1993; Heard 1999; Cruz *et al.* 2005; Cortopassi-Laurino *et al.* 2006). They can be seen foraging high atop rain forest trees and assist in the creation of forest diversity within Australia (Wille 1983). Though their role in pollinating native flora is well documented, their efficacy in horticultural and agricultural crops of Australia needs further study (Heard 1999).

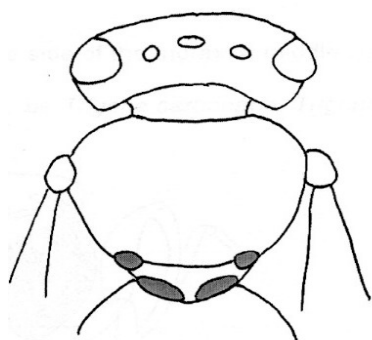
Colony populations of 100 to 100,000 have been estimated for different species of stingless bee (Bourke 1999). Some researchers have used brood volume (cm<sup>2</sup>) to estimate populations (Wille and Michener 1973) while others have used brood cell numbers (Roubik 1983; van Veen and Sommeijer 2000). Roubik calculated that the adult population would not exceed two thirds of the brood population. van Veen and Sommeijer (2000), when studying reproductive swarms, estimated brood populations of between four and a half thousand and just under fifteen thousand per colony. Few studies of this type have been conducted using Australian stingless bees. It is estimated that *A. australis* colonies consist of one queen, drones (males) and hundreds to thousands of female workers.

Stingless bee colonies, like the honeybee *A. mellifera*, reproduces perennially (Rayment 1935; Michener 1974). Workers raise brood, feed and care for its members (including the queen), build nest structures, forage for nectar, pollen and resin, ripen honey and defend against predators (Rayment 1935; Michener 1974; Dollin 1996c; Dollin 1996a; Dollin 1996b). However, unlike *A. mellifera*, stingless bees have a reduced and functionless sting and are, therefore, called “stingless” (Rayment 1935; Michener 1974; Wille 1983; Dollin 1996a; Heard 1999). As such, stingless bees must rely on different defence strategies from those of the stinging honeybee (Wittmann 1985).

As there has been minimal research carried out on *A. australis*, much of the subsequent information on biology and behaviour is based on published literature for other closely related stingless bees, particularly *Trigona* and *Plebeia* spp., from Australia and overseas.

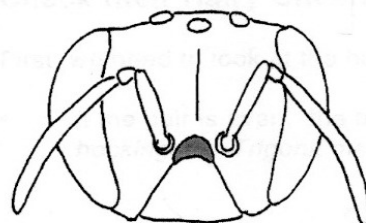
## 2.2.2 Morphology of the stingless bee

*Trigona* and *Austroplebeia* appear similar but on close examination have subtle defining differences. *Austroplebeia* can be distinguished from *Trigona* by differences in body colour and markings, thorax shape and nest architecture. *Trigona* is completely black whereas



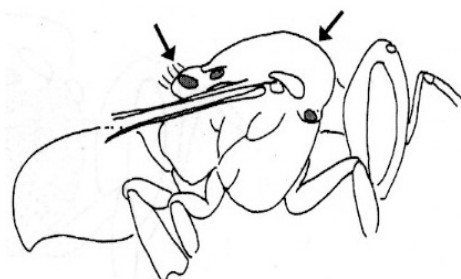
**Figure 8: Thoracic markings of *Austroplebeia* (Dollin 1997)**

*Austroplebeia* is black with cream to yellow markings on its face and the scutum of the thorax (Figures 8 & 9) (Dollin 1997). The shape of *Trigona*'s thoracic back rim is also more angular than *Austroplebeia*'s flatter thorax (Figures 10 & 11) (Dollin 1997).

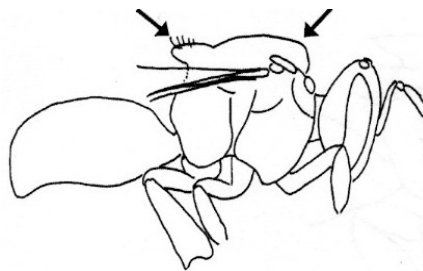


**Figure 11: Facial markings of *Austroplebeia* (Dollin 1997)**

Stingless bees have morphological adaptations which enable them to gather and safely store pollen whilst foraging on flowers (Rayment 1935). Like many bees, *A. australis*



**Figure 9: Thoracic markings and shape of *Austroplebeia* (Dollin 1997)**



**Figure 10 Thoracic shape of *Trigona* (Dollin 1997)**

accumulates fine granules of pollen on the, possibly electrostatic (Thorp 1979), branched hairs of their face, thorax and legs (Dollin 1997), as they forage. This is then gathered up, using brushes or combs (Thorp 1979), as the bee cleans herself and is packed into the corbiculae, or “pollen baskets”, found on the surface of the hind tibiae. This structure enables the bee to forage and collect large loads of pollen at a time (Rayment 1935).

A small amount of nectar is added to the pollen mix to aid in its packing. The stingless bee also uses the pollen basket to transport resin (Rayment 1935; Thorp 1979; Patricio *et al.* 2002) for use in the nest structure. In *Austroplebeia* the inner surface of the hind tibial keirottrichiate area is broad and almost reaches the upper margin of the tibia; this is not so for *Trigona* (Michener 2000).

In *Apis* the workers’ sting is an aborted ovipositor and therefore the drones have no sting (Rayment 1935). Stingless bee workers have an atrophied or vestigial sting (Rayment 1935; Heard 1999) and are, therefore, unable to sting. *Trigona*, as described by Rayment (1935), has short wings and with only five to six hooklets (hamuli), thus rendering it incapable of long distance flight. Its mouth parts include a long and hairy glossa which enables foragers to visit a variety of floral species (Rayment 1935; Wille 1983; Heard and Hendrikz 1993; Heard 1999; Heard and Dollin 2000).

### 2.2.3 The life cycle of the Australian stingless bee

*A. australis* starts its life as an egg, usually laid by the queen (Michener 1974; Dollin 1996a; Drumond *et al.* 1999; Michener 2000). Meliponinae queens mate only once and some are unable to remove the male genitalia, which is ripped out when mating occurs, until they return to the nest (Michener 1974; Palmer *et al.* 2002; Toth *et al.* 2004). The sperm is stored in their spermatheca and is used to fertilise most of the eggs that are laid (Michener 1974).

Fertilised eggs become females, either workers or queens, and unfertilised eggs become males, or drones. All male stingless bees are capable of mating (Dollin 1996a). Workers can develop ovaries (Drumond *et al.* 1999) but reproduction is usually inhibited by queen-produced pheromones (Michener 1974). While drones are predominantly produced by the queen a considerable proportion can be produced by laying workers (Koedam *et al.* 2005), although this is rare in Australian stingless bees (Rayment 1935; Michener 1974; Dollin 1996a; Drumond *et al.* 1999; Toth *et al.* 2004). Drummond and her colleagues showed that *A. australis* workers

were capable of laying abnormally large eggs but only small numbers were observed, with all recorded instances of worker eggs eaten by the queen (Drumond *et al.* 1999).



**Figure 12:** *A. australis* workers provisioning a brood cell ready for ovipositioning (Drumond *et al.* 1999)

The queen appears to inspect the progress of events. Finally the queen places her head into the cell, eats some of the larval food and then lays her egg on top of the mix. The cell is quickly sealed by one of the attending workers while the queen and the other workers move away (Drumond *et al.* 1999).

The queen lays her eggs in specially prepared ‘brood cells’. In *A. australis*, five or six workers are attracted to the newly constructed cell, where they signal the queen of the cell’s readiness. They ring the rim of the cell (Figure 12), while head-bobbing and vibrating their wings. Once the queen’s attention has been gained the workers begin provisioning the cell with larval food (Drumond *et al.* 1999). This is a mixture of honey, pollen and secretions from the hypopharyngeal glands (Michener 1974). Throughout this process, the queen

Once the cell is sealed the offspring remains encapsulated until it emerges as an adult (Michener 1974). The larva does not hatch, as such, but absorbs the egg capsule into its body as it develops (Michener 1974). The white larva develops and grows, eating the food provided within the cell. The amount of food provided to the developing larva determines whether the emerging bee will be a worker or a queen (Rayment 1935; Michener 1974; Dollin 1996a). Unlike *Apis*, stingless bees do not feed royal jelly to the potential queens, but instead place the egg in a larger cell with a greater amount of food (Rayment 1935; Michener 1974; Dollin 1996a).

Once the larva is fully mature, it spins a cocoon within the cell, to become a pupa. The initial cell wall, comprised of cerumen (see 2.3), is removed from the cocoon by workers and recycled within the nest (Michener 1974). The pupa eventually develops, through metamorphosis, into an adult bee. It emerges from its cocoon and is the same size as the other workers within the colony, although slightly lighter in colour (Rayment 1935; Dollin 1996a).

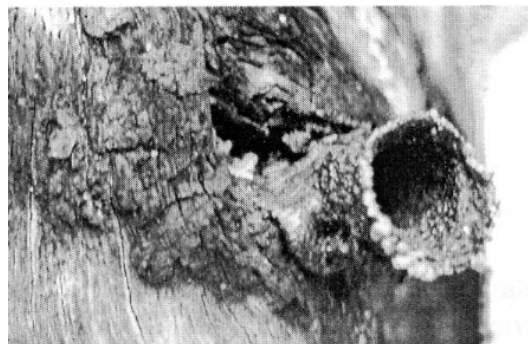
#### 2.2.4 Nest architecture of the stingless bees

Stingless bees secrete wax from their metasomal tergal glands and mix it with resin collected from trees (Wille and Michener 1973). In Australia resin is often collected from members of the

Casuarinaceae family (Rayment 1935) or from turpentine (*Syncarpia glomulifera*) (Dollin 1996c) but can be collected from any suitable plant (Wallace and Trueman 1995). Fresh paint has also been found to be a satisfactory substitute (Dollin 1996c). The mix of resin and wax, called cerumen, is used in nest construction (Rayment 1935; Michener 1974; Wille 1983; Dollin 1996c).

The nest entrance is often difficult to locate as it is usually only 10-12mm in diameter and is hidden in a crack or knot in the tree trunk (Dollin 1996c).

*Austroplebeia* builds a small resinous tube (Figure 13), approximately 6-10mm wide, which protrudes slightly from the side of the tree (Dollin 1996c). The



**Figure 13: Resinous entrance tube of an *australis* nest in 1996c)**

tube runs from the outside of the tree to the interior of the nest and may measure only a few millimeters or as long as one meter (Rayment 1935; Wille 1983; Dollin 1996c). Often the tube is built along the inside wall of the nest for some distance (Figure 14) (Rayment 1935; Wille 1983).

Once inside the nest, there are supporting and interconnecting cerumen structures throughout (Wille and Michener 1973). The strength of the supports and colour of the structures are governed by the proportion of resin to wax. The more resin, the harder, darker and stronger is the structure (Michener 2000). The cavity that holds the nest is often lined with plates of batumen, a rough, hard mix of resin and wax constructed into sheets. This helps to seal, waterproof and insulate the nest cavity (Dollin 1996c; Michener 2000).

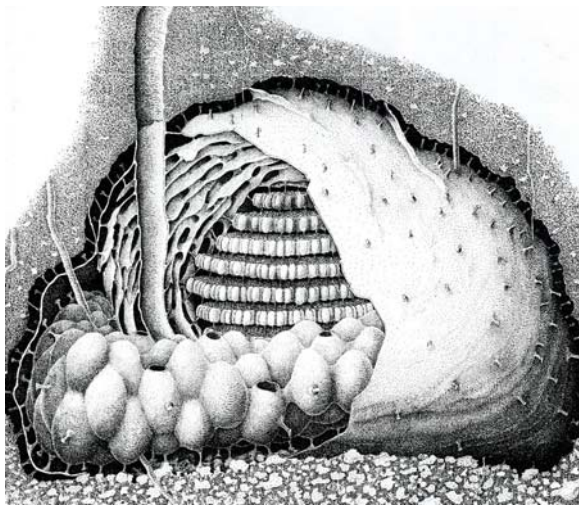


**Figure 14: Tube within nest, similar to that of *Austroplebeia* (Rayment 1935)**

Australian's *T. carbonaria* seals off any holes leading into the tree cavity with hard batumen plates. They then line the entire cavity with a batumen lining made of a thin layer of resinous material, to ensure no intruders penetrate their defensive barriers (Dollin 1996c).

Within the nest (Figure 15), stores of honey and pollen are held in pots constructed from soft cerumen, which is more easily crafted than is batumen. Again, *Trigona* use more resin in their mix, resulting in a thicker, darker cerumen than that of the *Austroplebeia* (Dollin 1996c). The pots measure approximately 9-16mm wide by 10-22mm high in a *T. carbonaria* nest (Dollin

1996c). As each clump of pots is finished and sealed they are covered with a layer of involucrum, also made from cerumen, which protects and insulates sections within the nest



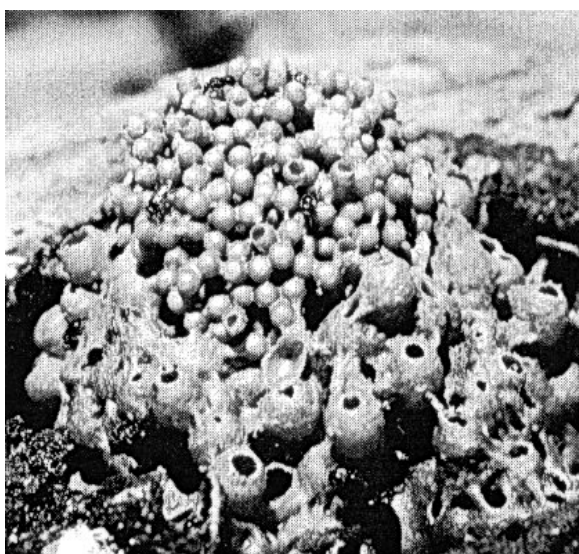
**Figure 15: A subterranean *Trigona* nest:** Showing entry tube (top), honey and pollen pots (bottom left), batumen casing (right), brood (centre), involucrum surrounding brood (top left) (Michener 1974)

(Rayment 1935; Michener 1974; Wille 1983; Dollin 1996c; Michener 2000).

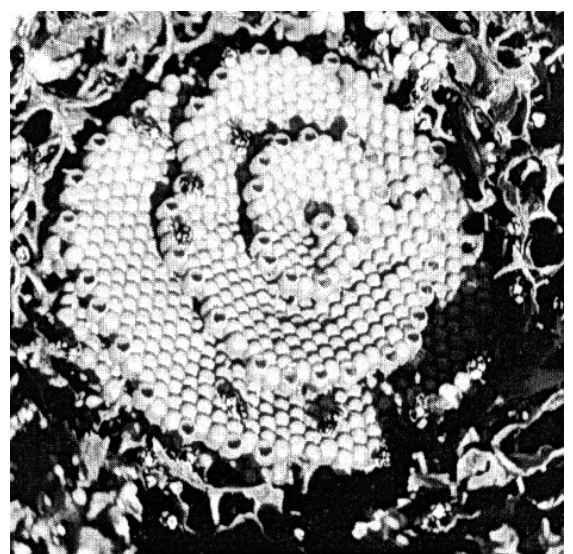
Brood comb is constructed from soft cerumen and are usually located towards the middle of the nest (Dollin 1996c). The brood comb architecture of the *Austroplebeia* is different to that of the *Trigona*. *Trigona* builds 3-4mm elongated, hexagonally shaped brood cells with

the openings facing upwards (Figure 17), with some building these cells in singly-layered, regular horizontal spirals. *Austroplebeia* builds

3-4mm diameter, spherical brood cells in irregular clusters (Figure 16), with the openings facing in any direction (Dollin 1997). These clusters can be surrounded by honey or pollen (Dollin 1996c). As each cluster of brood is completed it is covered with a thin layer of involucrum to protect and insulate the section of the nest (Dollin 1996c). It has been suggested that brood clusters, such as those constructed by the *Austroplebeia*, are an adaptation for habitation of small, irregular cavities within tree branches, which would not support the construction of brood comb (Pooley and Michener 1969). In Queensland, *Austroplebeia* nests have been found in tree branches, as much as seven metres in length (pers. com. R. Zabel October 2006).



**Figure 16: *A. australis* brood cluster** (Dollin 1996c)



**Figure 17: *T. carbonaria* brood comb** (Dollin 1996c)

### 2.2.5 Nest dynamics

The house keeping performed by the stingless bee is quite unique. They include “rubbish dumps” (Dollin 1996c) within their nests, where they stock-pile debris and faeces during inclement weather. Unlike *Apis* species, stingless bees are able to defaecate in their nests without the risk of dysentery (Rayment 1935) or *Nosema apis* (Czekonska 2000). This rubbish is removed by workers of various ages and is essential for the control of disease within the colony (Rayment 1935; Dollin 1996a). Other methods of disease control are the evaporation of nectar into more concentrated honey, the use of antibacterial resins (Velikova *et al.* 2000; Fernandes Jr *et al.* 2001) in the construction of the nest and food pots and the use of resins to seal over the bodies of invaders within the nest (Dollin 1996a).

Thermoregulation of the nest is important to ensure the brood does not get too hot in summer nor too cold in winter. Nest siting is an important component, as the thick trunk of a tree plays an integral part in insulation (Michener 1974; Wille 1983). *T. carbonaria* nests can be maintained at temperatures between 26°C and 28.5°C, while outside temperatures range from 10°C and 30°C (Dollin 1996a). This is made possible with the combination of the insulation properties of the tree, removal of hot air from the nest by wing fanning and the use of nest structures to take advantage of moisture loss and, therefore, evaporative cooling (Roubik 2006). As there is no evidence that Australian stingless bees can maintain temperature homeostasis (Wille 1983), insulation within the cavity is most important in protecting against heat and cold. It is thought that brood chamber temperature is maintained through the insulation properties of the tree, layers of batumen and involucrum plus the heat generated by the brood (Wille 1983). Added warmth may be generated when the bees gather together in clusters on the brood area and vibrate their flight muscles (Rayment 1935; Michener 1974; Wille 1983; Dollin 1996a), however, many stingless bees do not have the ability to generate warmth through clustering. Australian stingless bees are only found naturally in tropical and sub-tropical regions (Dollin 1996b) where colonies do not have to thermoregulate to survive against the cold temperatures (Michener 1974; Wille 1983).

Artificial nesting boxes are ineffective substitutes for tree trunks with regard to insulation, and stingless bee keepers need to be aware of the dangers of unstable weather when setting up hives (Dollin 1996a; Dollin and Heard 1999; Dollin *et al.* 2001).

### 2.2.6 Division of labour in stingless bee colonies

Once she emerges from her cocoon the new worker self-grooms and unravels her wings. She is fed her first drop of honey by a nursery worker (Dollin 1996a). She soon moves on to helping in the nursery with brood incubation and repair. Unlike *Apis*, young stingless bees are able to produce wax and begin working in the nursery soon after emergence, constructing and working brood cells (Cepeda 2006). Nursery work continues with cell construction and provisioning, feeding young adults and the queen plus cleaning. *Plebeia remota* (Holmberg) (van Benthem *et al.* 1995), constructs brood comb in batches of 50 cells at a time. The cells are sequentially provisioned by workers. Four to six hours is required to prepare and provision the 50 cells, into which the queen subsequently lays her eggs once the batch is completed. This process is performed each 24 hours. *A. australis* construct their brood cells successively and do not construct them in organized batches (Drumond *et al.* 1999). Additional in-hive duties include involucrum construction, nectar reception from foragers and subsequently, guard duty.

The final role of the worker is foraging for pollen, nectar and resin (Wille 1983; Dollin 1996a). Young workers do not have well developed mandibular glands to leave foraging scent trails (Wille 1983). Longevity is dependent on the amount of activity a worker performs and how quickly she is “worn out” (Michener 1974). The average lifespan of some stingless bee workers ranges from 24-50 days depending on the level of foraging and other work taking place (Wille 1983).

### 2.2.7 Colony reproduction in stingless bees

As social bees are often thought of as “super-organisms” (Moritz and Southwick 1992), reproduction relates to the duplication of the colony rather than the reproduction of individuals within a colony (Queller and Strassmann 2002; Seehuus 2006). In *Apis mellifera*, reproduction by swarming can coincide with colony crowding and/or the occurrence of favourable environmental conditions (Michener 1974). It is triggered by colony size and foraging conditions (Seeley 1985). The workers initiate queen cell construction over appropriate young female brood and restrict queen egg laying so that she is able to follow, when the reproductive swarm leaves. Scout workers guide the swarm to a new suitable nesting site (Seeley 1985). A number of queen cells, containing virgin queens ready to emerge, is left behind within the nest with at least 50% of the colony’s workers. The two colonies become completely independent of each other as soon as swarming is initiated (Michener 1974). Since nest-mate recognition in

*Apis* is environmentally determined, the workers of the two colonies does not recognize each other after a few weeks and will fight when they are encountered each other ((Bethe 1898) cited in (Pirk *et al.* 2001).

Stingless bee colony reproduction is performed in a more sophisticated way and the new colony remains dependant upon the mother colony for several weeks or even months after it is first established (Michener 1974; Wille 1983; Inoue *et al.* 1984; van Veen and Sommeijer 2000). When conditions are conducive, scouts leave the mother colony in search of suitable nest sites. Once located, workers begin cleaning the cavity, sealing cracks and constructing an entrance, often using materials removed from the mother colony (Michener 1974; van Veen and Sommeijer 2000). These workers commute to and from the mother colony taking resin, cerumen, pollen and honey to the new nest site (Michener 1974; van Veen and Sommeijer 2000). With such a dependency on communication between both colonies it is advantageous if they are only a short distance apart (van Veen and Sommeijer 2000). Stores can also be gathered from the local environment (Wille 1983; van Veen and Sommeijer 2000). Once the new nest is established the new virgin queen (gynae) and a swarm of workers leave the mother colony. van Veen and Sommeijer (2000) reported swarm arrivals of between two and 13 days from initial nest preparation. Inoue *et al.* (1984) estimated that approximately 30% of the original colony workers eventually remained with the daughter colony but observations by van Veen and Sommeijer (2000) estimated only 10% were recruited to the new colony. Soon after arrival of the swarm, a drone swarm appears outside the nest and the virgin queen takes flight to mate (Michener 1974; Wille 1983; van Veen and Sommeijer 2000). Most stingless bees are monandrous and only mate with one drone (Rayment 1935; Michener 1974; Green and Oldroyd 2002). Stingless bee queens may take two to seven days before egg laying commences (van Veen and Sommeijer 2000).

## 2.2.8 Stingless bee foraging and communication

Communicating the location of food and nest resources is essential for colony survival. Like *Apis*, stingless bees communicate in many ways. The most primitive communication involves pilot flights by scouts who directly lead new foraging recruits to the resource (Esch *et al.* 1965; Michener 1974; Aguilar *et al.* 2005). In Brazil, *Trigona* (*Scaptotrigona*) *postica*, *T.* (*Axestotrigona*) *tescorum*, *T.* (*Partamona*) *cupira* and *T.* (*Friseomelitta*) *varia* and *Melipona quadrifasciata* use sound within the nest (Esch *et al.* 1965; Wille 1983; Nieh and Roubik 1998; Aguilar *et al.* 2005) while *Trigona carbonaria*, *Melipona panamica* and *M. seminigra* use scent

trails leading from the nest to the resource (Wille 1983; Kerr 1994; Nieh and Roubik 1995; Dollin 1996a; Nieh 1998; Hrnčir *et al.* 2004). Overseas studies have shown stingless bees in the genera *Trigona* and *Melipona* can also communicate height, distance and direction of a resource (Nieh and Roubik 1998). The few studies carried out on Australian stingless bees have shown that *Trigona* species leave a trail of glandular secretions, leading from the resource to the colony (Dollin 1996a). In-hive activities such as trophallaxis and antennal tapping communicate resource type and quality (Sommeijer *et al.* 1983). There has, however, been limited research with *Austroplebeia*.

### 2.2.9 Defence mechanisms of the Australian stingless bee colony

The first line of defence for a stingless bee colony is the nest and its entrance. The nests are secreted in cavities in logs and trees. The entrances are reduced to the size of three or four bees wide and sometimes the entrance is closed with sticky resinous substances (Rayment 1935; Wille 1983; Wittmann 1985; Dollin 1996c). *A. australis* constructs a curtain of cerumen at the entrance of the nest (Dollin 1996c), which is erected at dusk to aid in the defence of the nest and also during inclement weather. It is torn down each warm morning and stored for reuse (Dollin 1996c).

Devoid of a stinger, Australian stingless bees rely on other defensive tactics such as biting, grappling and attacking *en masse* (Wille 1983). Animal intruders can experience dozens of tiny jaws attached to the inside of their ears and nose, their eye lids and even their eyeballs (Dollin 1996a; pers. com. M. Duncan 2006), ultimately being driven away from the bees' nest.

Insect threats are initially attacked by entry guards (Dollin 1996a), who release an alarm pheromone to recruit more defenders as necessary (Wille 1983; Wittmann 1985; Dollin 1996a). Potential intruders are met before (Wittmann 1985) and at the door of the nest and grabbed by several guard bees. They incapacitate the insect intruder by holding onto its wings and dropping to the ground, where it is wrestled to death (Dollin 1996a). Successful interlopers may meet their death by being entombed alive inside the nest structure. *Trigona* covers unwelcome visitors in a resinous substance, stored within the nest (pers. comm. P. Neumann, M. Duncan, R. Spooner-Hart, A. Dollin and D. Hoffman 2005, Dollin 1996a). *Austroplebeia* nests contain smaller stores of resin (pers. com. Dollin 2005), leading to concerns about their ability to effectively defend themselves against potential predators or parasites.

Natural predators of the Australian stingless bees include spiders (Dollin and Heard 1999), lizards (pers. com. M. Duncan 2005), cane toads (pers. com. Russell Zabel 2006) and humans (Cortopassi-Laurino *et al.* 2006). Parasites include syrphid (Diptera: Syrphidae) and phorid (Diptera: Phoridae) flies. These parasites are usually only a threat when a hive has been split, disturbed or damaged (Dollin 1998; Dollin and Heard 1999). They lay their eggs inside the hive or in cracks created by hive damage, and the fly larvae move around the nest, consuming the food stores (Dollin 1998; Dollin and Heard 1999).

## 2.2.10 Meliponiculture

Meliponiculture is the practice by which beekeepers reproduce stingless bee colonies, of various species, for profit. This profit may be in the form of honey, cerumen, resin, nucleus colonies, bushland or crop pollination, education, conservation of the species or just a hobbyist's pleasure (Heard and Dollin 2000). Cortopassi-Laurino *et al.* (2006) carried out a world-wide survey of communities that practiced meliponiculture and found this culture throughout South America, Central America and Mexico, as well as small areas of northern Australia. According to Cortopassi-Laurino *et al.* (2006), the ancient Mayans considered the stingless bees worthy of a place in their religious worship, including a God of honey in their ensemble of deities. Stingless bee honey has been used for medicinal purposes and is used in the treatment of gastro-intestinal upsets, ocular complaints, ulcers and wounds and coughs (Vit *et al.* 2004; Cortopassi-Laurino *et al.* 2006). The honey has a higher moisture content (30-42%) than *Apis* honey (18-20%) (Bijlsma *et al.* 2006), and requires pasteurisation or refrigeration to avoid fermentation (Cortopassi-Laurino *et al.* 2006); pers. com R. Zabel October 2006).

Limited research has been carried out on the chemical composition of the honey, pollen, and yeasts contained within stingless bee colonies (Sommeijer *et al.* 1983; Fernandes-da-Silva and Serrao 2000; Cortopassi-Laurino *et al.* 2006). Further work required includes; honey preservation post-harvest, sustainable reproduction of stingless bee colonies, education of farmers in the reduction of bee losses through correct pesticide use and correlation of all research material to enable efficient whole-community education and industry training (Cortopassi-Laurino *et al.* 2006).

In Australia, in 1984, the practice of managed meliponiculture was almost non-existent, but a survey conducted in 1998/99 showed considerable growth in its popularity, and it was predicted that meliponiculture would steadily increase over the next twenty to thirty years (Heard and Dollin 2000). Since then interest in stingless bee keeping has increased significantly, with

groups being established for the conservation of species (Cortopassi-Laurino *et al.* 2006) and small businesses that have developed methods for colony propagation for sale to hobbyists, and training of indigenous Australian communities in the tropics to propagate colonies and harvest hive products to promote sustainability and maintain a niche cultural industry (pers. com. R. Zabel October 2006).

Deforestation is impacting on the number and diversity of wild colonies in countries such as Malaysia (Samejima *et al.* 2004) and Brazil (Brown and Albrecht 2001; Cortopassi-Laurino *et al.* 2006).

### 2.2.11 Native and agricultural crop pollination by stingless bees

The stingless bees' ability to visit and pollinate, with floral constancy (Heard and Hendrikz 1993; Ramalho *et al.* 1994; Heard 1999; Hilario *et al.* 2000; White *et al.* 2001), a large variety of plants (Heard 1999) together with its low susceptibility to European honeybee pests and diseases (Delfinado-Baker *et al.* 1989) makes the exploitation of these bees an attractive agricultural and horticultural activity. The fact that eusocial stingless bees are perennial (Wille 1983) ensures the presence of pollinators throughout the year (Heard and Hendrikz 1993). This is particularly important in year-round greenhouse crop production (Amano 2004; Malagodi-Braga and de Matos Peixoto Kleinert 2004). They are successful foragers within the confines of a glasshouse (Heard 1999; Can-Alonzo *et al.* 2005; Cruz *et al.* 2005) and, being stingless, they are also less harmful to the humans tending the crops (Heard 1999).

Although some stingless bee species are capable of 1.5km flight distances (Roubik and Aluja 1983) *Trigona* and *Austroplebeia* prefer to forage only one to two hundred metres from their nest (Dollin 1996a), to a maximum of 500 m (Bartareau 1996). The distance a stingless bee is prepared to fly depends on how attractive the resource is (Heard 1999) and the relative size of the bee (Michener 1974).

Of the 1000 or more plant species cultivated in the tropics, approximately 250 are compatible with stingless bee pollination (Heard 1999). Many economically significant, cultivated crops originate from regions where *Apis* species do not naturally occur (Heard 1999). In recent years artificial hives have been introduced into macadamia orchards, with good results (Heard 1999). Other crops to have benefited from stingless bee pollination include choko, coconut, mango, carambola, Amazon tree grape and plants from the Myrtaceae family, a dominant family in Australian forests (Heard 1999). Their contribution to forest biodiversity is considerable;

studies showed that nine out of thirteen Australian orchid species were pollinated by stingless bees (Adams and Lawson 1993). Stingless bees also assist in the pollination of strawberries, squash, coriander, the medicinal “belly ache bush” of Brazil and India, Indian shot and avocado (Heard 1999; Malagodi-Braga and de Matos Peixoto Kleinert 2004).

## 2.2.12 Conclusion

The nests of both *Austroplebeia* and *Trigona* contain honey, pollen, wax, adult bees and bee brood, all of which equate to the food of preference for the African SHB (Ellis *et al.* 2002b). It is this commonality with *Apis* species that is of concern to scientists, bee keepers and conservationists alike (Neumann and Elzen 2004). The SHB, outside its native range, has devastated *Apis* honey bee colonies. The threat that SHB imposes on *Apis* populations also threatens agricultural and horticultural industries. Crops such as stone fruit, nuts and seed oil require insect pollinators, many of which are *Apis* bee spp existing in managed and unmanaged colonies (Radford *et al.* 1979; Langridge and Goodman 1985; Heard 1999; Goodman *et al.* 2001). The existence of a bee pollinator, with an ability to defend against infestations of SHB, could, potentially, fill the pollination gap produced by a reduction in *Apis* populations. The possible threat to stingless bees from this newly introduced hive predator has led to this research project. The vulnerability of Australian stingless bees to SHB infestation required study to be better understood.

This broad aim became the rationale for the conducting of my studies, which are documented in this thesis.

### 3 Aims

#### 3.1 Objectives

- To observe and elucidate normal in-hive behaviour of the Australian native bee *Austroplebeia australis*, including its defensive behaviour.
- To develop a methodology for the non destructive study of in-hive behaviour of the Australian native bee, *Austroplebeia australis*, and its interaction with all life stages of small hive beetle, *Aethina tumida*.
- To better understand the defense mechanisms of the Australian native bee, *Austroplebeia australis*, against hive invasion, with particular reference to infestation by small hive beetle, *Aethina tumida*.
- To assess the efficiency of the Australian native bee, *Austroplebeia australis*, to incapacitate or remove all life stages of the small hive beetle, *Aethina tumida*, introduced into hives
- To investigate the behaviour of *Aethina tumida* in hives of *Austroplebeia australis*, including their evasive and defensive behaviour

#### 3.2 I investigated these aims, based on the following premises:

1. *A. australis* is capable of being fed an in-hive supplementary pollen as a protein source when external conditions are unsuitable for foraging
2. Although *A. australis* is a tropical species, it can be maintained under extreme conditions of heat and cold experienced at Richmond NSW, in a controlled-environment house
3. An observation platform can be connected to a *A. australis* hive, allowing observation but without changing their normal behaviour
4. *A. australis* has developed defence mechanisms against colony-invading predators, involving methods other than stinging
5. While eggs and larvae of *A. tumida* may be unable to effectively defend themselves against *A. australis*, adult beetles should be able to survive attacks. Thus:

- a. Exposed eggs of *A. tumida* will be destroyed in a *A. australis* colony
- b. Exposed larvae of *A. tumida* will be destroyed in a *A. australis* colony
- c. Exposed adult *A. tumida* will not be destroyed in a *A. australis* colony

## 4 Materials and methods

### 4.1 General Materials and Methods

#### 4.1.1 Experimental limitations

There were a number of limitations which restricted the nature and extent of my studies. These are discussed below.

- Stingless bee colonies can be challenging to propagate and as such it can be difficult and expensive to source supplies. Reduced availability and a restricted budget dictated that this study would be carried out using only four hive replicates.
- The nature of the tropical stingless bee, *Austroplebeia australis*, dictated that the hives be housed in controlled temperature rooms and that they be given supplementary feeding to maintain them through the cold winter of Richmond, NSW. This meant that this Honours project had to be run over at least a three semesters (18 month) period.
- The procured stingless bee colonies were in a very weakened state, due to being split only weeks before transport to Richmond, therefore the winter period needed to be used to build hive strength, to provide appropriate strength colonies to test against SHB.
- It had been anticipated that the SHB could be easily reared just prior to the experiments being conducted, but it soon became apparent that they could not. Hence, provision of SHB in sufficient number for experiments and thus the timing of these studies became reliant on other researchers, who were busy with their research and their time constraints.

### 4.2 Background studies and methodologies

#### 4.2.1 Initial hive preparation

In February 2006 four OATH (Original Australian Trigona Hive) boxes (Dollin and Heard 1999), each containing a colony of *Austroplebeia australis*, were obtained from a commercial stingless bee producer in southern Queensland (Russell & Janine Zabel, Gatton, Queensland, Australia, 4343) and transported to a garden estate at Blaxland NSW (33°45'S, 150° 36'E), where they were allowed to settle for ten weeks.

Each hive was removed from its cardboard box and the hive entrance was opened and fitted with a 5cm long, silicone tube ( $\varnothing$  12.5 mm), to aid monitoring of hive flight activity (Figure 18). Each hive lid was opened and a Perspex sheet (20cm x 28cm x 3mm) was placed on the top of the hive, facilitating regular colony observations while keeping disturbance to a minimum.

The colonies were transported to the University of Western Sydney's, Hawkesbury campus (33° 36' S, 150° 47' E), where the experiments were to be conducted. "Hive Activity" was monitored and recorded from February to December 2006.



**Figure 18: Workers orienting to new location and modified hive entrance**

#### 4.2.2 Observation of stingless bee behaviour

This work was conducted to gain a greater knowledge and understanding of the normal behaviour of the stingless bee *A. australis* in the hive, and to do this by the least disruptive method possible. This was designed to provide a useful basis on which changes in behaviour, due to the introduction of SHB into the nest, could be better assessed and evaluated.

From the time of initial receipt of the *A. australis* hives behavioural observations commenced. When the hives were first opened bee behaviour was noted. Behaviours recorded included nest mate fighting within the same colonies, nest site orienting, hygienic behaviour and debris removal. Hive activity was observed for each hive, for a minimum of 20 minutes per hive, each week with observations noted, recorded, photographed or videoed throughout the ten months study period.

Observation and photographic records were taken, using a Nikon D50 Dslr camera, fitted with either an AF-S Nikkor 18-55mm or an AF Micro Nikkor 60mm lens, of each hive, through the Perspex lid, to monitor colony growth parameters such as brood volume, honey and pollen storage and increase in nest structure. Other photographic recording included forager communication (Michener 1974; Sommeijer *et al.* 1983; Wille 1983), worker trophallaxis (Hrncir *et al.* 2006), entrance guard behaviour (Michener 1974; Dollin 1996a) and house keeping activities (Dollin 1996a). A JVC Hard Disk Camcorder was used to capture footage of behaviour such as provisioning and oviposition processes (POP) and queen feeding (Wille 1983; Drumond *et al.* 1999), brood cell, pollen and honey pot construction, sealing of hives and

OP breeches and worker/queen interaction (Michener 1974; Dollin 1996a). Other activities, that could not be captured on film included nectar ripening, pollen packing, nectar and honey regurgitation (Dollin 1996a) and entrance fanning were noted and described. Defensive actions were also witnessed against possible intruders including the syrphid flies, ants and in one incidence of a honey bee, *A. mellifera*. These activities appeared similar to that reported by other authors, so was considered “normal”.

### 4.2.3 Seasonal bee flight and foraging activity

*A. australis* flight behaviour during the experimental period was recorded and compared with seasonal changes in temperature and day length as well as short-term weather conditions.

Hive entrances were observed for flight activity approximately weekly, between 1200 and 1600h (Eastern Australian Standard Time), for flight activity. The number of bees returning to each hive entrance over a two minute period was counted and recorded. The number of returning bees carrying pollen was counted at the same time. Observations of in-hive activity such as queen sighting and active brood building were also noted. Volume of supplementary honey mix taken per week and if pollen had been removed from provided stores. Weekly activities are shown in Table 1.

Data was collected from data loggers on a regular basis. These data were used and cross checked with the Bureau of Meteorology (BOM), from the weather station at Richmond RAAF Air Base [http://www.bom.gov.au/climate/averages/tables/cw\\_067033.shtml](http://www.bom.gov.au/climate/averages/tables/cw_067033.shtml). Additional data was computed with the aid of the Geoscience Australia website, [http://www.ga.gov.au/bin/gazmap\\_sunrise?placename=richmond&placetype=A&state=NSW](http://www.ga.gov.au/bin/gazmap_sunrise?placename=richmond&placetype=A&state=NSW) which allowed computation of sunrise and sunset at the specific longitude/latitude.

With the knowledge that *Austroplebeia* and *Trigona* do not forage at temperatures  $<18^{\circ}\text{C}$  (Heard and Hendrikz 1993) pers. comm. A. Dollin November 2005), the period of time that temperatures were  $\geq 18^{\circ}\text{C}$  (Appendix A) during daylight hours was calculated and used to estimate the available daily foraging hours, to compile monthly averages. These were compared with the bee flight activity and hive weights, which had been monitored throughout the year.

**Table 1: Weekly monitoring of behaviour parameters**

Activities monitored from 26/02/06 to 01/12/06	Treatments from 04/05/06 to 15/08/06
Number of foragers entering hive in 2 minutes ( <i>n</i> )	Amount of honey consumed (ml)/week
Number of foragers entering hive with pollen in 2 minutes ( <i>n</i> )	Pollen replaced ( $\sqrt{x}$ )
Active brood building ( $\sqrt{x}$ )	
Queen sighted ( $\sqrt{x}$ )	
Room temperatures (°C)	
External temperature (°C)	
Weather conditions (description)	

#### 4.2.4 Initial preparation of insectary

Because *A. australis* is a tropical species, suitable conditions had to be developed to enable the colonies to be maintained and studied outside of their native range.

This was achieved by housing the four experimental colonies in two controlled temperature (CT) rooms. These rooms were insulated, cooled by thermostatically-controlled air conditioners, and heated, when required, with a small fan heater with a thermostat. The walls, ceiling and floors of the rooms were thoroughly cleaned and disinfected prior to commencement of the studies. Thermostats were set to maintain the room temperatures at  $26 \pm 2$  °C.



**Figure 19: Hive setup inside CT rooms**

Clean, laminated, 45 cm deep particle board shelves were placed in the rooms. The underside of the shelves held 35 watt florescent tubes for lighting. A 12 volt, 50 watt halogen lamp (Figure 19) was used to illuminate studies as required. The edges of the floors of each room were sprayed with an insecticidal surface spray containing 2.0g/kg Cypermethrin, 3.5g/kg Tetramethrin, 30g/kg (s)-Hydroprene (Raid Surface Spray®, S.C Johnson & Son Pty

Ltd. Lane Cove, NSW Australia) to reduce the incidence of ant infestation. Spraying was repeated every three to four months.

Care was taken not to spray the whole floor. During some experiments bees escaped and they would drop to the floor when the room lights were turned off. This enabled them to be gathered up and released outside.

To allow the bees foraging access outside the rooms, one hole was drilled in each CT room external wall, using a round ( $\varnothing$  40 mm) saw. A 30 cm length of black polythene pipe ( $\varnothing$  30 mm) was fixed into the wall and siliconed in place. This provided an external access for bees to freely forage as required (Figure 20).

“Tinytag” temperature data loggers (Hastings Data Loggers, Port Macquarie NSW, 2444, Australia) were placed in each room to

accurately monitor conditions hourly. Another data logger was placed outside the CT rooms in the apiary, to record external weather conditions. It was placed inside the top of a 600ml PET plastic bottle to protect it from the elements and was located 20 m from the CT rooms within the UWS apiary grounds, one metre above the ground and shaded by shrubs. The CT room data loggers provided information that helped maintain optimum temperatures in the rooms. The external temperature data were used to generate external temperatures (which was compared with BOM information), to correlate with day length and foraging activity.

Hive entrances were only 1.5 metres apart, so large coloured shapes were numbered, laminated and placed above each hive entrance (Figure 20) to facilitate orienting the bees to their hives (Michener 1974).

#### 4.2.5 Development of observation platform

Prior to undertaking behavioural studies of *A. australis*, a non-disruptive and non-destructive observation had to be developed.

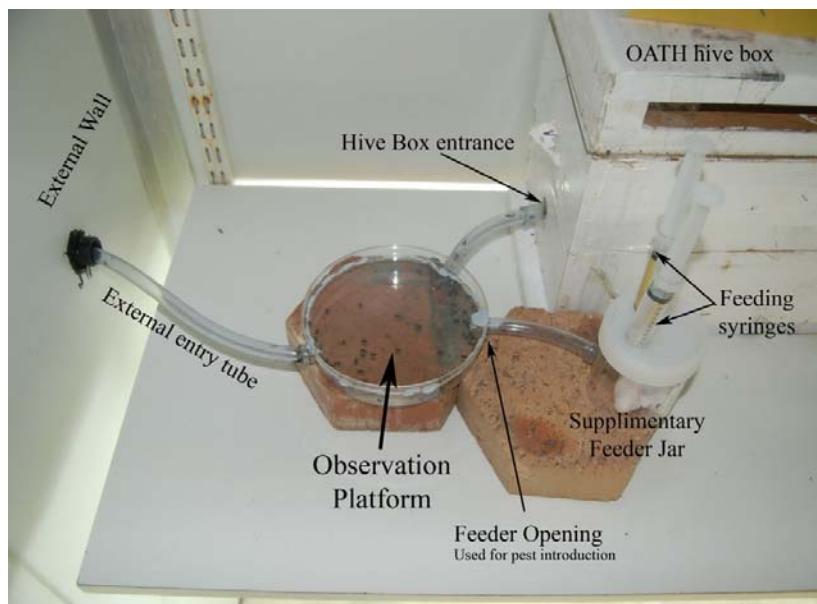
Previous studies conducted with *Trigona carbonaria* and SHB had resulted in the destruction of a number of hives and the death of colonies (pers. comm. P. Neumann, M. Duncan, R. Spooner-Hart, A. Dollin and D. Hoffman 2005, Dollin 1996a). My priority was to preserve the *A. australis* colonies and to build up colony strength, to high populations at the beginning of the experimental period. This was to ensure the colonies were at optimal health and that studies would be carried out using hives that were representative of natural stingless bee colonies, whether either managed or wild. In addition, I needed to be confident that I was able to clearly observe hive behaviour without causing any abnormal bee activity associated with the methodology.



**Figure 20: External hive entrance. Showing poly pipe, silicone entrance tube, hive number**

A bee observation platform, modified from a unit developed at the University of Sydney (Dr William Hughes, pers. comm.) was installed for each hive. This area would serve as an observable extension to the main hive and was designed to enable multiple experiments with minimal risk to colony health and behaviour.

Each Observation Platform (OP) was constructed around a Petri dish ( $\varnothing$  15 cm). Three holes were melted into the side of the Petri dish base and one into the side of a (150ml) specimen jar. A “Hotery” butane micro torch (1300°C) was used to heat the plastic and then a short piece of ( $\varnothing$ 10 mm) silicone tubing was pressed



against the heated area. This was repeated until the plastic gave way and the tubing could be twisted into place. Once the plastic had cooled the tube was removed and later replaced with the tube that would sit in the hole permanently. Care was taken not to break the plastic Petri dish, as it became quite brittle after heating. Two holes were drilled into the specimen jar lid, to fit the hubs of two 5ml syringes, and cotton wool was placed in the bottom of the jar, to reduce the incidence of drownings due to honey spills. One end of a 1 metre length of silicone tubing ( $\varnothing$  10 mm) was fed through the external poly pipe and the other end was connected the OP. Two more 10 cm lengths of ( $\varnothing$  10 mm) tube were connected from the OP to the hive box, and the OP to the specimen (feeder) jar. A thin roll of adhesive putty (Blu Tac) was set on the rim of the Petri dish (OP) base and the inverted lid was sealed in place (Figure 21).

**Figure 21: Experimental setup with hive and observation platform**

#### 4.2.6 Modifications to the original set-up

Follow up evaluation of the set-up the following day revealed a flaw in the design. A gap remained between the two external tubes and as some foragers returned they entered the CT rooms by squeezing in between the silicone tube and the poly pipe: there were 93 foragers found on an early occasion on the floor of the Hive 2 CT room. The external entrance tubing was sealed into place, inside the poly pipe, with adhesive putty. While worker losses occurred

occasionally during the study period this was kept to a minimum by constant monitoring and modification of the hive set-ups.

Modifications to the feeder unit were also necessary: the bees had difficulty accessing the honey while the 5ml syringe hubs were intact. To increase the accessibility of the honey mix, the hubs were trimmed and secured in the jar lid holes with the aid of additional adhesive putty.

Vibrations emanating from the CT rooms' cooling systems caused the feeder jars to vibrate and the honey mixture to drip from the, now modified, syringe hubs, causing potential bee drownings. To reduce the vibrations and movement of syringes, a ( $\varnothing$  22 mm) round saw was used to cut one hole in the lid of each feeder jar, to fit the barrel of a 20ml syringe, with adhesive putty used to fix these in place. The pollen storage caps were also modified by bending their edges in to reduce honey dripping into them. The cotton wool which was originally placed in the base of feeder jars was replaced by scraps of cloth when it was observed that the bees spent considerable time and energy removing tiny balls of cotton wool via the OP and disposing of it at the front entrance in warm weather.

In October, the cooling system in the CT room housing Hives 3 and 4 broke down and the room over heated; the recorded temperature on the data logger in the room was  $> 42^{\circ}\text{C}$ . while this caused a set-back in these hives, they were, surprisingly, not severely affected.

OPs had to be replaced during the experimental period. This was due to reduction in visibility through the lid, and damage had also occurred over the five months. A week was allowed from the time of replacement to the time of experimentation to ensure the bees time had to settle into the new structure. Replacement was carried out using a newly prepared Petri dish, as previously described, and a temporary OP, with only two holes in the side. The temporary OP was placed on top of the old OP and the tubes from the hive box and the entrance were disconnected from the old OP and immediately connected to the temporary OP. The holes of the old OP were closed with a piece of cotton wool. Bee escapes were minimal during this procedure. The temporary OP ensured the bees could continue foraging and carrying out their normal duties while the new OP was being prepared. The old OP was taken outside to a table set up adjacent to the hive entrance, then opened to release the enclosed bees. The contents of the old OP were carefully removed, using a No: 10 scalpel blade and handle and long, blunt forceps, and placed inside the newly prepared OP. Contents consisted of pollen pots, which were easily relocated, honey pots, which posed more of a problem and were easily damaged, and cerumen structure, which were repositioned in approximately the same orientation within the new OP. All possible traces of resin and cerumen were transferred to the new OP. The rim of the new dish was topped

with a thin strip of adhesive putty and the lid was put in place. The completed unit was fixed into position between the hive box and entrance tube. Finally, the temporary OP was taken outside and the enclosed bees release close to their hive entrance. This procedure was carried out for all four hives. The old OPs were left near the hive entrances so the bees could gather the honey and resin residue left behind. On one other occasion only the lids of the OPs were replaced to improved experimental observation.

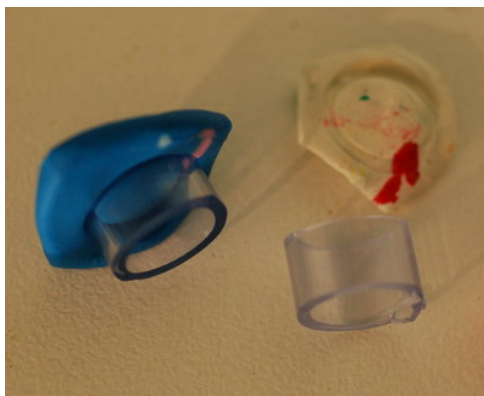
Bee escapes were unavoidable during some experimental procedures. To reduce mortality during bee escapes, the lights of the CT room were turned off and the CT room door opened. Bees dropped to the floor and began walking toward the daylight. Extra illumination was provided with an 80 watt flood lamp outside the CT rooms. As bees walked out the door they were swept up and released outside. It was important to keep the floors clean and the area free of spider webs.

#### 4.2.7 Consolidation of colony strength

Prior to conducting investigations with SHB invasion, it was important to build up the experimental hive colony strength. As previously discussed, the *A. australis* colonies had been recently split before their arrival (Dollin *et al.* 2001) from Queensland. Moreover, they had only a limited time span (ten weeks) to build up food reserves before the cooler weather set in, which limits foraging of this tropical species (Heard and Hendrikz 1993; Hilario *et al.* 2000). Therefore, each colony was provided, on a weekly (and later twice weekly) basis *ad libitum*, with a supplementary food supply of an *A. mellifera* honey/water mixture (1:1 v/v ratio) using 20ml syringes and finely ground (using the apiary spice grinder), irradiated honeybee, *A. mellifera* -collected pollen, placed in recycled Stelvin screw caps ( $\varnothing$  30 mm). The pollen was replaced every two to three weeks.

The pollen filled caps were placed in the bottom of the feeder jar. It was difficult to replace the pollen, and therefore to monitor the consumed amount accurately, because when the lids were removed from the feeder jars, dozens of bees would crawl out. It was considered that the casualty rate was too high to remove the lids every week and as a result, excess pollen was provided to ensure an adequate supply for the two to three week period.

A honey/water mix (1:1 v/v) was made up in batches, with surplus mix being stored in a refrigerator. For each colony, a 20ml syringe was filled, the empty syringe, in the feeder jar, was carefully eased out until almost fully removed. The full syringe was quickly slotted in to



**Figure 22: Capped tubes used to close feeder opening**

replace the empty syringe to minimise the number of bees escaping through the small hole. Syringes were replaced when plungers became stuck (approximately fortnightly), and in any case, monthly.

When supplementary feeding was no longer required the feeder jars were removed from the OP and the opening

was closed with a plasticine-capped 3 cm length of silicone tube ( $\varnothing$  10 mm) (Figure 22). This opening was subsequently used for the introduction of SHB.

#### 4.2.8 Rearing and sourcing SHB

All life stages of SHB (eggs, larvae and adults) were required for the investigations.

On September 19 2006, adult SHB were collected from two naturally infested *A. mellifera* colonies, located at the UWS apiary, using a standard aspirator for insect collection. A combination of the SHB rearing methods described by Neumann *et al.* (2001) and Haque and Levot (2005) was used in rearing attempts. Twenty adult SHB were placed in a plastic rearing container (35 cm x 20 cm x 100cm), lined with moistened paper towel. The container had a sealable lid, with a modified with a window (10 cm x 8 cm) of nylon mesh for air flow. Two ribbed, 180 ml plastic drinking cup, placed one inside the other, were provided for oviposition (SHB oviposit in cracks and crevices in hives (Ellis et al. 2004a)). The food source provided was sugar crystals and ground pollen (1/2 teaspoon of each), as described by Haque and Levot. Each was placed in a ( $\varnothing$ 5 cm) Petri dish, inside the rearing container. The rearing container was stored in an incubator at set to 29°C with a large bucket of water to increase humidity. The temperature and humidity were monitored with data loggers. Temperature was maintained at 27±2° C but the humidity varied between 58-95% RH.

Successful attempts to rear SHB of all life stages was limited. Oviposition did not occur on the structure provided. Two small clusters of eggs were found on the bottom of the food dishes. The SHB food was replenished and container misted with water to raise humidity because periods of low humidity in the incubator increased SHB mortality. The sugar and pollen were mixed into a paste, using a few drops of water, and placed in a ( $\varnothing$ 5 cm) Petri dish and a small piece of *Apis* brood comb was introduced into the rearing box. Even so, this methodology was ineffective in

rearing sufficient cohort numbers for the detailed investigations, although all preliminary studies were conducted using SHB from this original rearing.

Further supplies of SHB eggs, larvae or adults were sourced from scientists with greater experience in this field. This, however, meant that there was a reliance on others to source SHB specimens, and experimental schedules were timed around beetle availability.

The preliminary studies were carried out using the limited number of successfully reared SHB life stages. These studies were undertaken to assess how *A. australis* might react to the introduction of a small number of SHB, prior to introduction of larger numbers under more formal experimental conditions. As a result of these preliminary studies, modifications to planned procedures were made.

### 4.3 Investigations on response of *A. australis* to introduction of different life stages of SHB

#### 4.3.1 Response of *A. australis* colonies to introduction of SHB eggs

##### 4.3.1.1 Objective

To observe and evaluate the ability of *A. australis* colonies to detect and remove introduced SHB eggs from the nest.

##### 4.3.1.2 Methodology

Introduction of all life stages of SHB, into the OPs via the feeder openings were carried out with a minimum of disturbance to the bees or the structures within the OP (Figure 23). The studies on SHB eggs commenced when the OPs had been *in situ* for 18 weeks. The inside surfaces of all the OPs

had become covered in a film of resin, honey and wax and visibility was greatly reduced. The lids of the OPs were therefore replaced at the time of the first egg introductions.

Preliminary studies showed that the bees ate introduced SHB eggs. To obtain pictorial evidence of this, SHB eggs were introduced onto the new lids of the OP, for the first set of studies. The



**Figure 23: OP showing feeder opening to be modified for SHB introduction**

limited number of eggs available at this time required the studies to be conducted within a short time span.

Groups of SHB eggs (N=30) were randomly separated from clusters using a moistened fine camel hair paint brush. The eggs were transferred to the inside of a new OP lid, which was then substituted for the previous lid. Bee activity was observed and photographed. Egg numbers were monitored and recorded at intervals of 30, 60 and 90 minutes. OPs were checked for any remaining eggs after 24 hours. A total of 30 eggs were introduced into each of the four OPs.

For subsequent studies 30 SHB eggs were placed on a 10 mm x 25 mm piece of coloured plastic sheet and introduced into the OP via the feeder opening, using long forceps. Egg numbers were recorded at intervals of 30, 60 and 90 minutes. This was carried out for all four colonies. At the conclusion of the first of these studies, the plastic sheet with any remaining eggs was carefully removed via the feeder opening, using the long forceps. This procedure was repeated an hour after the completion of the previous one. Any eggs remaining at the end of this study were left in the OP to determine whether the bees would remove these eggs or cover them with resin during the subsequent 12 hours.

#### 4.3.2 Response of *A. australis* colonies to introduction of SHB larvae

##### 4.3.2.1 Objective

To observe and evaluate the ability of *A. australis* colonies to detect and remove introduced SHB larvae from the nest.

##### 4.3.2.2 Methodology

Preliminary studies were conducted to determine how the stingless bees would behave when a SHB larva was introduced into a colony. A single, mature SHB larva, approximately 11 mm length x 1.5 mm diam., was collected from the rearing box and introduced into the OP of Hive 2, and observed for two hours. Hive 2 had less hive structure within its OP and so bee and SHB behaviour would be observed more easily. A single mature larva was later introduced into the OP of Hive 1. Both OPs were observed for a further three hours. These preliminary studies indicated that *A. australis* were unable to effectively dispose of large, mature larvae. This situation was also unrealistic, as colonies would initially be faced with eggs or small larvae, not

mature ones. Thus smaller and more uniform sized larvae were sourced for the detailed investigations.

A single control treatment was set up to determine whether any behaviour of the bees observed towards introduced SHB larvae was a true defensive behaviour or merely a hygienic/ cleaning



**Figure 24: Three day old larva inside capped tube**

behaviour (i.e. removal of a foreign object). Three pieces of latex shaving, measuring approximately 10mm x 1mm (similar dimensions to SHB larvae), were placed inside the OP of Hive 2 and the behaviour of the bees was monitored for two hours.

As the latex shavings had not been removed after two hours, they were left in the OP, to determine how long it would take the bees to remove them.

Ten, uniform three-day old, 8 mm x 1 mm, laboratory reared larvae were randomly selected and placed into a 20 mm long ( $\varnothing$  10 mm) closed ended silicone tube (Figure 24). The open end of the tube was inserted into the feeder opening and the larvae entered the OP. This was undertaken for all four experimental hives. Bee removal of larvae was monitored for each OP and the time of each removal was recorded, over a 15 minute period. OPs were subsequently physically checked after two hours, to ensure that no larvae remained in the structure. This procedure was repeated for each hive.

### 4.3.3 Response of *A. australis* colonies to introduction of SHB adults

#### 4.3.3.1 Objectives

Two separate investigations were conducted to assess the response of *A. australis* to adult SHB in hives. In the first instance, adult beetles were introduced into the OP (as occurred with eggs and larvae), and in the second they were introduced to the hive entrance.

In the latter investigations, which more closely mimicked the likely situation in nature, the additional objectives were

- to observe and assess the ability of *A. australis* entrance guards to halt the progress of SHB adults into the hive entrance.

- to observe and assess the ability of *A. australis* to recruit additional guards to the hive entrance as a result of a potential threat posed by adult SHB
- to observe and assess the behaviour of *A. australis*, and its effectiveness, to detect, remove or incapacitate SHB adults invading the hive entrance

#### 4.3.3.2 Introduction

As stingless bee colonies are often found in tree hollows (Rayment 1935; Wille and Michener 1973; Mutsaers 1991; Heard and Hendrikz 1993; Dollin 1996c; Cortopassi-Laurino *et al.* 2006) they can inadvertently be damaged during tree lopping, land clearing and even storms, leaving them vulnerable to predators and parasites. Like-wise, the practice of splitting artificial hives for propagation or honey harvesting purposes can increase the incidence of infestation of hives by syrphid flies (Dollin and Heard 1999; Dollin *et al.* 2001) or SHB (Duncan, M. 2005 pers. comm. November; Luttrell, R. 2006 pers. comm. March; Alonso, C. 2007 pers. comm. January). The introduction of SHB adults into the OP of the *A. australis* experimental hives simulated the invasion of a damaged hive.

Most colonies of stingless bees, however, exist in sealed, intact log hives or in well managed artificial hives and are better able to defend against predators and parasites (Rayment 1935; Wille and Michener 1973; Michener 1974; Wille 1983; Dollin 1996a; Dollin and Heard 1999; Heard and Dollin 2000). The introduction of SHB adults to the entrance of the experimental hives enabled assessment of *A. australis*' ability to defend its hive entrance. In addition, the ability of the colonies to effectively deal with adults that penetrated into the hive proper was investigated.

Preliminary studies indicated that the bees could not incapacitate the adult beetles quickly and that the latter could pose a threat to the health of the entire colony unless they were restricted to the OP. A 3 mm-holed,

metal mesh was cut to fit on the end of a plastic 150ml specimen jar.

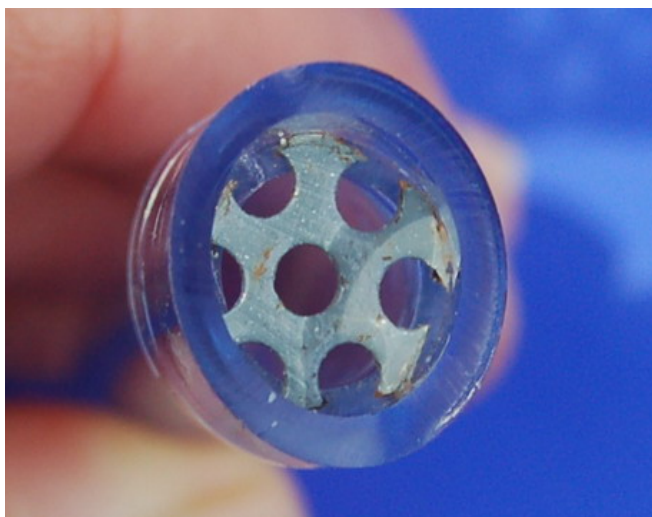
Twenty randomly selected adult SHB were placed inside the jar and the mesh lid was then



**Figure 25: Comparative sizes of adult SHB and *A. australis***

closed and taped in place masking tape. The jar was placed, on its side, in the SHB rearing box, together with a pollen and sugar food supply (see section 4.2.8). The box was closed and left over night. Although the size of the SHB adults varied slightly (Figure 25) only the smallest (< 3 mm diam) of the beetles were able to escape from the jar, through the mesh, to access food. Future experiments subsequently eliminated small (< 3 mm diam.) specimens of adult SHB.

A piece of 3 mm-holed metal mesh ( $\varnothing$  12.5 mm) was cut to fit across the end of the OP tubing, to prevent SHB entering the hive proper. A 2 cm long ( $\varnothing$ 12.5 mm) piece of silicone tubing was heated in boiling water and eased over the end of the smaller tubing and mesh. The modified end of the tube fit snugly inside the hive box entrance (Figure 26). Bees were recorded, using the camcorder, and the number of bees passing through the modified entrance was compared to the number of bees passing through an unmodified piece of tubing. The number of bees entering



**Figure 26: Hive box modification**

and exiting the hive was approximately the same for both tubes, therefore, normal hive activity could take place during the time the hive entrance modifications were in place.



**Figure 27: Sticky substance on SHB**

In the preliminary study one adult SHB was observed coated by the bees, in a clear, sticky substance (Figure 27) but its assessment with a sugar refractometer found it contained no sugars.

#### 4.3.3.3 Methodology

For the introduction of SHB adults into the OPs, three randomly selected beetles were introduced into each of the four OP, on October 24 2006. SHB movement, ejection or incapacitation was monitored for a period of six hours. The final location of any beetle confinement within the OP was noted. All the OPs were inspected 24 hours after SHB

introduction. Their lids were removed and any remaining SHB were located, photographed and removed from the OPs. This experiment was repeated three days later, on October 27, 2006, but in this case the number of adults introduced was increased to five for each OP. The OPs were left undisturbed for 48 hours before being examined.

For the introduction of SHB adults into hive entrances adult beetles were collected from a number of *A. mellifera* hives, naturally infested with SHB at the UWS apiary, and were then stored in a rearing box, in the shade. Larger beetles were chosen to ensure they could not pass through the hive box mesh if they evaded guard bees. Five SHB adults were randomly removed at one time and held in a 150ml specimen jar. One beetle was removed from the jar and introduced into the front entrance of the first hive. The times of beetle introduction and ejection were recorded. If the SHB successfully broke through the guard bee defences, a period of 5 minutes was allowed before the next beetle introduction. If the SHB was ejected by the guard bees, the time was noted and a new SHB was introduced immediately. This process was carried out for each of the four experimental hives. The experiment was repeated a further two times, so that a total of 15 adult SHB were introduced into the entrance of each of the four hives within a 24 hour period. Approximately 60 hours after the final introduction the OPs were opened and the adult SHB were located, counted and photographed.

This study was repeated once more, so that a total of 15 adult SHB were introduced into the entrance of each of the four hives within a 24 hour period. At the conclusion of the second study the OPs were replaced, with all structures within the original OP carefully transferred into the new ones. A time period of three days was allowed to elapse to ensure the OPs were resealed and the bees had become accustomed to the new situation before any further studies were conducted.

The modifications to the hive box entrances were removed at the conclusion of these studies.

#### 4.3.4 *Austroplebeia* resin curtains as a barrier to SHB adult entry

##### 4.3.4.1 Objective

The objective of this investigation was to evaluate the effectiveness of *A. australis* resin curtains in reducing adult SHB entry into stingless bee hive entrances.

#### 4.3.4.2 Introduction

*Austroplebeia* spp are known to erect curtains of resin over their hive entrances, at night or during cold or inclement weather (Dollin 1996c). These investigations aimed to harvest curtains and determine their effectiveness against SHB intrusion. Curtains had been observed at the entrance of the hives (Figure 28) when they were sited in the garden estate prior to the experimental period.



**Figure 28: Resin curtain at entrance**

#### 4.3.4.3 Methodology

A 3cm length of silicone tubing ( $\varnothing$  7 mm) was placed inside the existing tubing at the front entrance of each of the four hives. The tubes were held together with plasticine to prevent them from moving. Five days later the tubes were inspected for traces of resin or presence of whole curtains. The weather was inclement, with fine drizzling rain, the ambient temperature was 13°C and it was just before dawn, supposedly ideal conditions for curtain production. However, no curtains had been constructed. The tubing was left *in situ* and inspected of a morning, twice a week, for two weeks. During the entire investigation period in the CT rooms, there was no sign of resin stores on the inside edge of the entrance tubes and as a result, this study was terminated.

## 5 Results

### 5.1 Background studies and colony and observation platform consolidation

#### 5.1.1 Observation of *A. australis* behaviour

When the experimental hives were first opened at the garden in Blaxland NSW (Section 4.2.1), following shipment of more than two days, > 20 instances were observed where nest-mates emerged fighting each other. This behavior can be described as groups of bees flying out of the hive entrance and immediately grasping each other in flight and then dropping to the ground. These “fights” contained from two to five bees locked together in a writhing ball. Most of the fighting groups remained locked together and died where they fell (Figure 29).



**Figure 29: Ball of at least three bees locked together after opening hive**

Hive house-keeping activities were observed from the first day after hive arrival, and were carried out at various times of the day. During the cooler seasons, workers were observed removing debris during the one or two warmer hours in the afternoons, between 13.00 and 15.00 EAST. Bees walked out of the entrance tube, holding debris in their mandibles and took flight, with the debris commonly dropped within half a metre of the entrance. While the hives were located in the CT rooms, during cold or inclement weather, the bees placed debris from the hive box in small piles within the OP. These piles disappeared within a couple of hours, when weather conditions became suitable for flight. Loose debris, such as cotton wool, paint brush bristle or latex shavings, within the hive was either removed within one to two days or resinned securely in place inside the hive. Bees were observed removing seeds from the Cadaghi tree (*Eucalyptus torelliana*), a tree indigenous to *A. australis*' native range, throughout the experimental period. Stock-piles of these seed were also stuck to the walls of the OATH box but were never incorporated into the hive structure

Construction of resin curtains was observed during the ten weeks the hives were kept outside, at Blaxland NSW (Section 4.2.1) (Figure 28), but not at any time during the investigative period. The resin curtains were torn down and stored just inside the rim of the entry tube when the

weather was warm. Once the hives were moved to the CT rooms, curtains were not seen again. Curtain erection coincided with the construction of involucrum sheets over brood clusters and the reduction and, in some cases cessation, of brood production. In-hive activity was low when viewed through the Perspex lid. Bees were never observed clustering *en masse*, over the brood area.

Communication between foragers and other workers was observed as the returning foragers entered the front entrance tube, and also as they moved further along the tube into the OP. Communication behaviour included trophallaxis (Michener 1974; Dollin 1996a; Ellis et al. 2002c) between forager and in-hive worker, antennal contact and wing beating (Wille 1983). Nectar exchanges also occurred between two or three bees and responses from workers varied between a passing communication to an excited and eager trophallactic exchange: this trophallactic communication lasted between one to 25 seconds. Workers returning to the hive box, after encountering adult SHB, would communicate with other workers for up to 25 seconds. There was also communication between workers and the queen, not associated with POP (see below). This included antennal beating, head-bobbing and nipping at the queen's wings and legs.

Provisioning and oviposition processes (POP) and queen feeding were frequently observed throughout the investigative period when active brood production was in place and was accessible to viewing. POP activity was recorded on video footage on two separate occasions. Workers were seen preparing a single brood cell, using soft, thin pieces of cerumen, and building it up from the brood cluster. Cells were often seen at varying stages of construction but a complete construction was never witnessed. Once a cell was constructed and ready for provisioning, workers attracted the queen's attention, by head-bobbing, antennal contact and wing beating. With the queen in attendance, four or five bees took from 1:43 minutes to seven minutes to provision the prepared cell with brood food, with the queen occasionally inspecting the cell. Finally the queen inspected the provisioned cell, appeared to eat some of the food within the cell and then lay an egg on top of the food. A single worker immediately sat in the cell and, using a rotating motion, began to seal the cell operculum, taking approximately six minute to complete this task.

The nest was constructed by in-hive workers carrying small ( $\leq 1\text{mm}$  long) strips of cerumen in their mandibles and adding them to existing structures. Different structures developed under different external environmental conditions. Cool conditions stimulated construction of involucrum sheets over the brood area and some of the honey and pollen stores. In Figure 30,

for example, the area marked (a) is the involucrum sheet which covers previously sealed brood cells, plus some food stores, while the area marked (b) is newly constructed brood, produced in the first week of hive introduction into the CT rooms.



**Figure 30: Different nest structures (a) involucrum (b) new brood**

Honey and pollen pots were built and filled continually and structural pillars and connections were constructed as the nest grew. There was no batumen lining constructed in any of the nests.

Newly emerged bees, which were pale and fluffy (Dollin 1996a), were observed moving around the brood area and occasionally one would hang onto nest structure with its mandibles, grooming its recently unfolded wings and body with its legs. Feeding of newly emerged bees by nurse bees was also observed

Entrance guard behaviour was observed weekly when forager counting took place, and was closely observed during the introduction of SHB. There were normally two to four guards at the entrance, and when the hive entrance was approached for inspection, the bees reacted in a timid manner, quickly pulling back, deep into the entrance tube.

Wing fanning was observed frequently as external temperatures increased, with the first incidence observed in early spring. Bees lined up in the entrance tube, in a straight line, facing away from the hive, and fanned their wings vigorously. The incidence of fanning appeared to increase after the hive box modifications were in place (section 4.3.3.2) with, in one case, 12 bees fanning in a line.

Forager gathering of resin and its storage within the nest was observed throughout the experimental period, with one experimental hive (Hive 4) apparently more active at this task. Within two weeks of the hive establishment in the CT rooms foragers had gathered large stores of resin and formed it into a cerumen wall (1 cm at the highest point) around the edge of the newly attached OP (Figure 31). With the onset of winter, and weather less conducive to foraging, the workers began to harvest resin from the OP wall, eventually resulting in holes in this structure.



**Figure 31: Cerumen wall constructed in OP 4**

While the OPs were being disassembled and inspected for SHB adult (4.3.3.3) the bees demonstrated their defence behaviour against larger (e.g. human) invaders, with this procedure conducted just outside the hive entrances. The OPs contained a large number of bees, but soon after I opened the chamber, the number of bees in flight increased further. Hundreds of bees buzzed around my head and face, depositing resin on my hair, and occasionally nipping my ears with their mandibles. After working with the OPs for one and a half hours the ends of my entire hair of had been resinned together, similar to hair lacquer.

Ripening of nectar took place near honey stores. Individual bees were observed rolling a bead of nectar in and out of their mouths, working the fluid with their proboscis. During spring, particularly if the weather was inclement, hundreds of bees were observed working nectar within a hive.

### 5.1.2 Development of observation platform and colony consolidation

On May 4, 2006, the hives were transferred from the garden location at Blaxland NSW to the CT rooms at UWS Hawkesbury. Prior to this, the colonies had begun to “shut down” their nests in preparation for winter. Resin curtains and involucrum sheets were constructed to coincide



**Figure 32: Workers sealing OP in first hours**



**Figure 33: OP entrance modification**



**Figure 34: Honey pots in OP**



**Figure 35: Long honey pot structures**

with a fall in night time temperatures (to 6°C). However, after the relocation of the hives into the CT rooms, which removed the temperature extremes to which the hives were exposed, colony activity increased.



**Figure 36: Hive 2, uncluttered OP**

commenced inside the OP (Figure 34) and in three months these grew into long beads filled with ripening honey (Figure 35).

By the time the SHB experiments commenced, all four OPs had some nest structure developed within them. One hive (Hive 2) had built entrance modifications but no honey pots, and because of ease of observation within this structure, many of the preliminary studies were conducted using OP 2 (Figure 36).

The bees consumed only small amounts (1.5 – 5 ml per hive) of the honey mixture in the first ten days but when the cold weather commenced, foraging ceased, and there was a



**Figure 37: Hive 1, 4 May 2006, Day 1 in CT rooms.**

corresponding increase in consumption of the

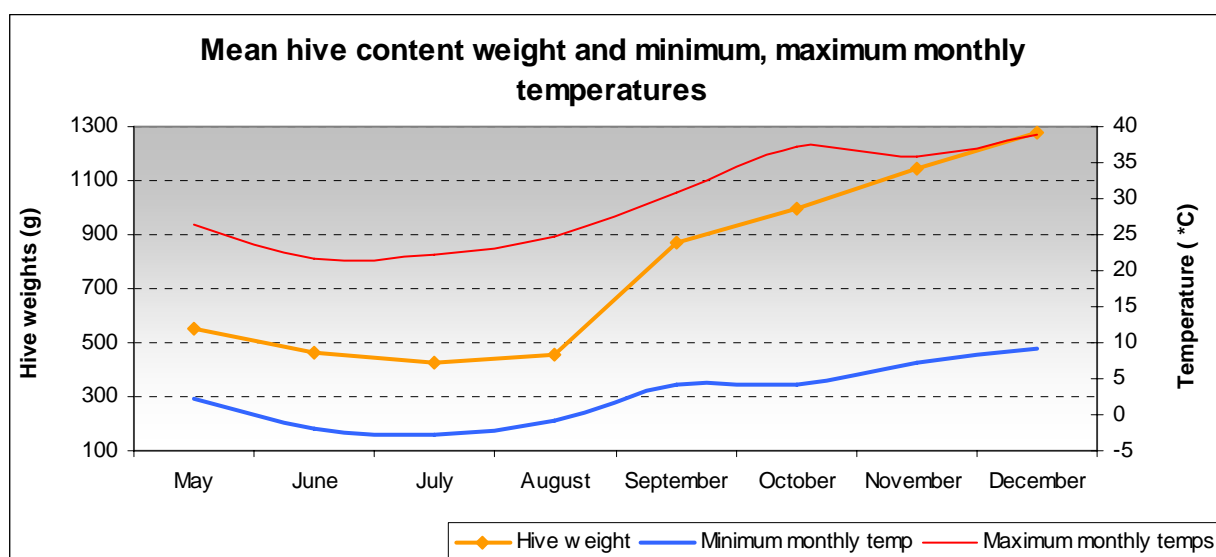
food supplements, with a mean of 40ml of honey mixture per week per colony being consumed. Monthly photographs of hive structures recorded an increase in brood growth through out the winter period. Quality of the images reduced as the hive structure advanced toward the Perspex lid (Figure 38) and as a result, progress photography ceased at the end of August, 2006. By this time all colonies were actively building brood. Figures 37 and 38 show the comparative



**Figure 38: Hive 1, 22 August 2006. Reflective surface impeding photographic quality**

increase in brood over a 16 week period. Note, in Figure 37 the small cluster of brood is covered with an involucrum sheet. This photograph was taken on the first day the hives were set up in the CT rooms.

Monthly hive weights recorded a decrease in mean contents weight of 17% over the four coldest months but with increasing external temperatures and corresponding flight activity mean hive contents weight had increased by 90% by the end of September (early spring) (Figure 39).



**Figure 39: Mean hive contents weight compared with minimum and maximum temperatures**

In early spring, as ambient temperatures and day lengths increased, the colonies began foraging regularly, with pollen packing evident in some hives. The bright yellow colours of freshly packed pollen in Hive 4 can be observed in Figure 40.

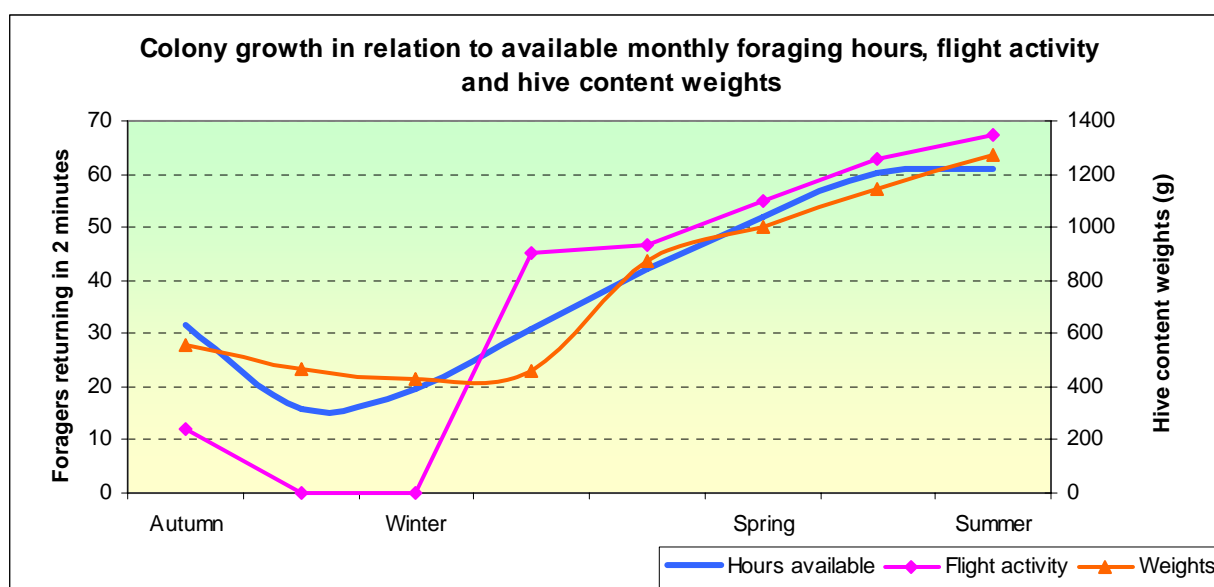
### 5.1.3 Flight activity

Weekly flight activity was monitored and the correlation between flight activity and pollen collection by the foragers was assessed. As the data were normally distributed, it was analysed untransformed. There was strong correlation (based on Pearson Correlation) between the total number of flights and the number of flights with pollen collected ( $r=0.714$ ). There was also a significant linear regression ( $P<0.001$ ), described by the equation  $y=0.174X-1.29$  (See Appendix B for data analysis).



**Figure 40: Freshly packed pollen pots**

With an increase in external temperatures and an increase in daylight hours the flight activity of all colonies increased. There was a corresponding increase in the mean weight of the hive contents, namely honey, pollen, resin and brood, which is presented in Figure 41.



**Figure 41: Colony growth compared to available foraging hours, flight activity and hive weight changes**

## 5.2 The response of *A australis* to introduction of different life stages of SHB

### 5.2.1 Response of *A australis* colonies to introduction of SHB eggs



**Figure 42: Inspecting eggs**

Following the introduction of SHB eggs into the OP, the behaviour of the bees was inquisitive and methodical, and there was no apparent urgency to remove the eggs. Upon their discovery, the eggs were inspected for several seconds by the workers, using their front legs and antennae (Figure 42). In Figure 43 a worker is observed scooping up an egg with her front legs and, holding the egg steady with her abdomen; she then began to eat the egg. Sometimes workers fought over the eggs, whereas on other occasions the bee who discovered the eggs bundled up two or three eggs at a time and quickly ate them before other bees arrived. The worker in Figure 44 approached the eggs with more caution,

Following the introduction of SHB eggs into the OP, the behaviour of the bees was inquisitive and methodical, and there was no apparent urgency to remove the eggs. Upon their discovery, the eggs were inspected for several seconds by the workers, using their front legs and antennae (Figure 42). In



**Figure 43: Eating egg**

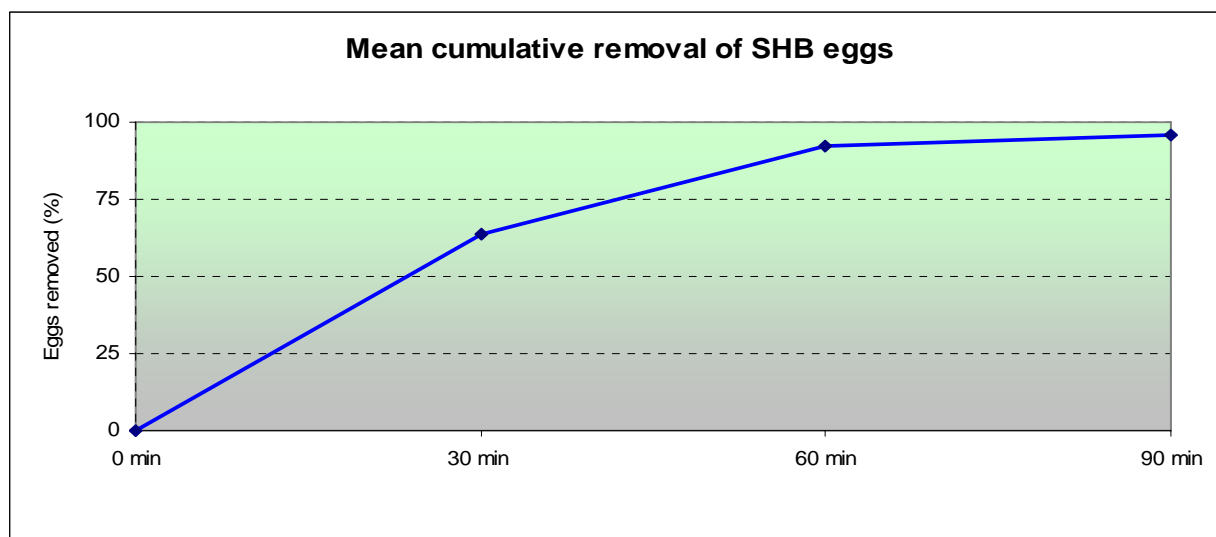
arming itself with a resin droplet before inspecting the objects.

During the third egg introduction in Hive 4, five eggs had resin droplets placed on them within 30 minutes and they remained resinned throughout the 90 minute observation period. These eggs were left in the OP over night and had been removed within the 18 hours which had elapsed since the previous observation.



**Figure 44: Worker armed with resin**

The mean cumulative percentage of SHB eggs removed, consumed or resinned for all replicates is shown in Figure 45. Approximately 50% of the eggs were consumed or resinned within the first 30 minutes. Destruction of 90 to 97% of eggs occurred within 90 minutes of their introduction.



**Figure 45: Mean cumulative percentage of SHB eggs removal over a 90 minute period**

### 5.2.2 Response of *A australis* colonies to introduction of SHB larvae



**Figure 46: Several bees wrestling with mature larva**

Preliminary studies on mature larvae showed the bees were unable to effectively eject large larvae. Figure 46 shows the comparative size of a mature larva and worker bees. Soon after introduction of the larva into the OP the bees inspected and attacked them but were unable to maintain a grasp on the writhing larva. The bees continued to attack the

larva with constant biting, pulling and other harassing behaviours for over an hour. It appeared to be that the bees could not coordinate defence strategy between each other: for instance, one bee was observed dragging the larva toward the front entry tube, but then it was dragged back by another bee. Four times during the observation period, bees were seen fighting each other and leaving the larva to crawl away from its attackers. Eventually this SHB larva was so severely injured that it ceased to move around the OP but it was not killed or removed from the hive during the five hour observation period. The larva was dead but still in the OP after 24 hours.

Bees were, however, able to pick up three- old larvae and carry them out through the front entry tube (Figure 47). Occasionally, bees were observed fighting over a larva and tug-o-war would ensue (Figure 48). This behaviour occurred most frequently in one hive (Hive 3) resulting in a lower larval removal rate. However, larval removal in this hive was more efficient during the second SHB larvae introduction period. Generally, the bees were efficient at removing the small



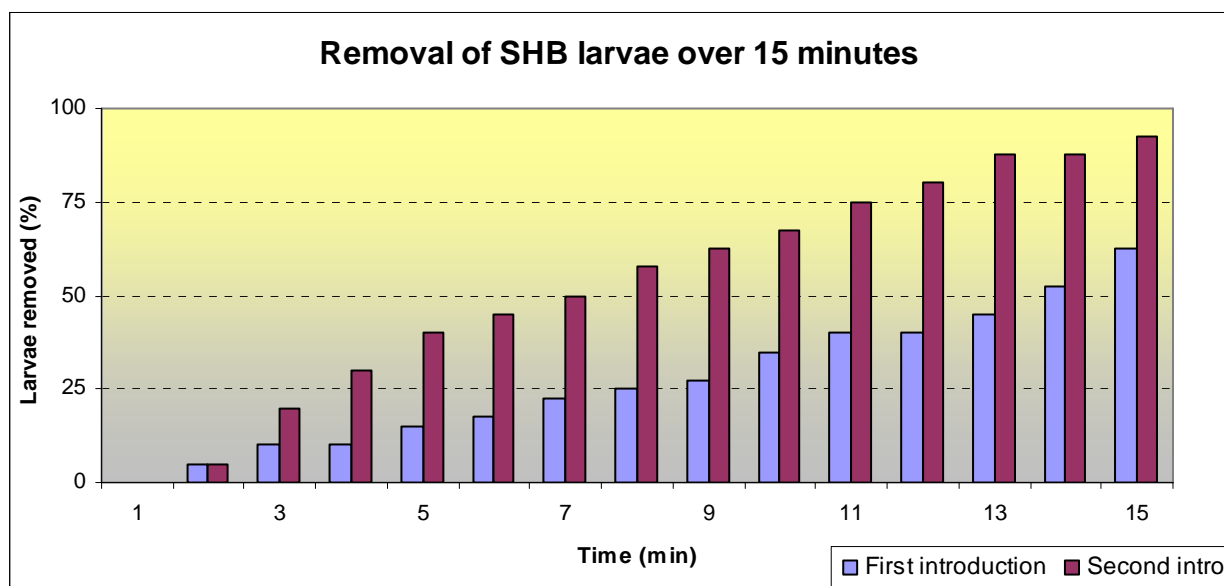
**Figure 47: One on one, effective removal of smaller larva**



**Figure 48: Inefficient tug-o-war behaviour**

larvae from their nest, especially when they worked independently.

During the first treatment only a mean of 62.5% of larvae were removed from the four OPs by the end of 15 minutes following introduction. In the second treatment there was an increase in larval removal, with 92.5% removed after 15 minutes (Figure 49). These data were subsequently analysed to compare differences in SHB larval survival between these two introduction periods.



**Figure 49:** Larval removal during first introduction compared to second introduction period

Kaplan-Meier survival analysis was used to examine the differences in the survivorship responses between treatments. The time taken for 10 to 90% ( $LT_{10}$  to  $LT_{90}$ ; respectively) of the test subjects to be killed (or removed) was estimated by observation. The observed  $LT_{10}$  to  $LT_{90}$  (Table 2) were the first time sample in which cumulative knockdown was  $\geq 10\%$  to  $\geq 90\%$ . Standard errors were calculated for each of these values. The significance of differences between treatments in the survival curves was determined using the log-rank (Mantel-Cox) test. The null hypothesis tested was that the mortality of test subjects was the same for all treatments. Significance was assessed using the unadjusted, comparison-wise error rate of 5% ( $\alpha = 5\%$ ). There were statistically significant differences between treatments in the survival curves for larvae ( $\chi^2 = 14.843$ ,  $df=1$ ,  $p < 0.001$ ). The lethal time deciles with their standard errors are shown in Table 2.

**Table 2:** Lethal time deciles for larvae. Values are the time (min) to achieve particular percentage mortality. Standard errors are given in parentheses.

Treatment	$LT_{10}^*$ (min)	$LT_{20}$ (min)	$LT_{30}$ (min)	$LT_{40}$ (min)	$LT_{50}$ (min)	$LT_{60}$ (min)	$LT_{70}$ (min)	$LT_{80}$ (min)	$LT_{90}$ (min)	$LT_{100}$ observed (min)	Log- rank test
First attempt	3 (1.42)	7 (1.98)	10 (1.51)	11 (2.32)	14 (0.90)	15 (-)	Not reached	Not reached	Not reached	Not reached	a
Second attempt	3 (0.42)	3 (0.51)	4 (0.73)	5 (1.03)	7 (1.27)	9 (1.31)	11 (1.10)	12 (1.01)	15 (-)	Not reached	b

$LT$ , Lethal time:  $LT_{10}$  = first time in which  $\geq 10\%$  of larvae were knocked down. (-) No variation. Treatments with the same letter do not differ significantly from each other.

### 5.2.3 Response of *A. australis* colonies to introduction of SHB adults

In-hive behaviour and interactions between SHB adults introduced directly into the OPs are described in the following section.



**Figure 50: Ejected SHB covered with resin globules**

Fortification procedures began as soon as SHB adults were introduced into the OPs. The bees showed immediate interest in the intruder. In several cases the SHB appeared unwilling to be there and tried to escape. Some of the more rapid ejections resulted from the SHB trying to leave the hive. At times, however, bees hindered the SHB's escape and drove it back into the OP.



**Figure 53: Attack increases in number. Bees were able to flip SHB**



**Figure 51: Bee riding SHB out of entrance tube**



**Figure 52: Initial attack with small numbers**

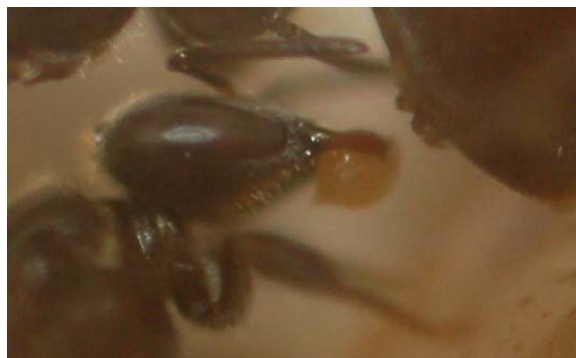
Ejected SHB were often covered with sticky globules of resin. The beetle in Figure 50 was still able to walk out of the hive entrance but was unable to fly, due to its resin coating. As resinned beetles crawled around the outside of the entrance tube the guard bees acted aggressively and bit the potential intruder, ensuring it remained outside, and at a safe

distance from the entrance. Sometimes a bee would “ride” the SHB out of the exit, biting the intruder as it hung on (Figure 51).

Within the OP, the SHB was initially attacked by five to six bees. Immediate defensive actions by the bees included riding, grappling and biting *en masse*. This was followed by the addition of resin globules which were stuck to the back of the SHB. Small globules of resin can be seen on the back of the SHB in Figures 52. With increasing time from the beetle introduction the number of attacking bees increased (Figure 53), with up to 45 bees attacking a SHB at any given time. Often, as the SHB pushed its way around the OP, it brushed attacking bee from its back by running under hive structure but the bee attacks continued unabated. Bees grabbed onto the SHB where ever they could get a hold but the “turtling” defences of the SHB limited their ability to grip.



**Figure 54: Hive structure with holes created by harvesting resin**



**Figure 55: Resin globule used in armory**

The resin armory defence was “backed up” by pieces of cerumen, harvested from the hive structure (Figure 54)

and glued onto the body of the SHB with the globules of soft resin (Figure 55). The bee on the right in Figure 56 can be seen holding pieces of cerumen while the bee on the left, armed with resin on its hind leg, holds onto the beetle.

Most successful beetle incapacitation was achieved when the bees were able to get it wedged



**Figure 56: Using resin (bee on the left) & cerumen pieces (bee on the right) to incapacitate adult SHB**



**Figure 57: Wedging adult SHB under structure**

into or under some part of the hive structure (Figure 57). This enabled them to build layers of cerumen around the beetle, incorporating it into the existing structure. Once the beetle was immobilised, the number of bees involved in the attack reduced, with only four or five bees continuing the entombment. Bee attendants frequently checked the entombed SHB, even several hours after it had been rendered harmless.

The behaviour of the bees changed throughout the experimental period. Upon introduction of



**Figure 58: Entombed bee (right) with adult SHB (top left)**



**Figure 59: Entombed adult SHB, still moving when removed from OP**

the SHB adults the bee activity increased and they immediately began harassing the beetle.

Their activity within the OPs continued to increase with time, and within four hours the colony became highly active. Wing fanning within the entrance tube was observed and bees were recruited from the main hive, with numbers doubling in the OP. As the activity and the number of bees increased some of the bees began fighting each other, balling and grappling for no apparent reason. There were two incidences where a bee was entombed along with the SHB (Figure 58).

Twenty-four hours after the initial adult introduction, the bees were busy but calm. The



**Figure 60: Single bee in attendance, after 24 hrs**



**Figure 61: Decapitated SHB, after 48 hrs**

entombed SHB still had guards in attendance (Figure 60). When the entombed SHB were removed from the OP, after 24 hours, the majority of them were still alive (Figure 59) although totally incapacitated and of no threat to the colony. However, those that were removed 60 hours after introduction were dead and, in some cases, decapitated (Figure 61)

Of the 32 SHB adults introduced directly into the OPs, 59% were removed and the remaining 41% were entombed, within six hours. The shortest removal time was five minutes and the quickest incapacitation time was 45 minutes. Removal and entombment times are shown in Table 3.

**Table 3: SHB adult ejected or entombed after introduction into stingless bee colonies (OP)**

<b>Adults introduced</b>	<b>Adults ejected</b>	<b>% ejected</b>	<b>Removal time (min)</b>	<b>% Ejected/entombed</b>	<b>Entombment time (min)</b>
32	19	59	5 – 240	100	45 – 350

Behaviour of guard bees towards the introduction of SHB adults, at the hive entrance, is described in the following section.

The number of guard bees at the entrance was low at the beginning of this experiment with only two or three bees standing at the rim and an additional two or three being just visible behind the front line (Figure 62). With the introduction of SHB adults the number of guards increased and the beetle ejection rate also improved with this increase (Figure 63).



**Figure 62: Small number of entry guards during initial introduction**



**Figure 63: Increased entry guard numbers**



**Figure 64: Guard armed with resin globules**

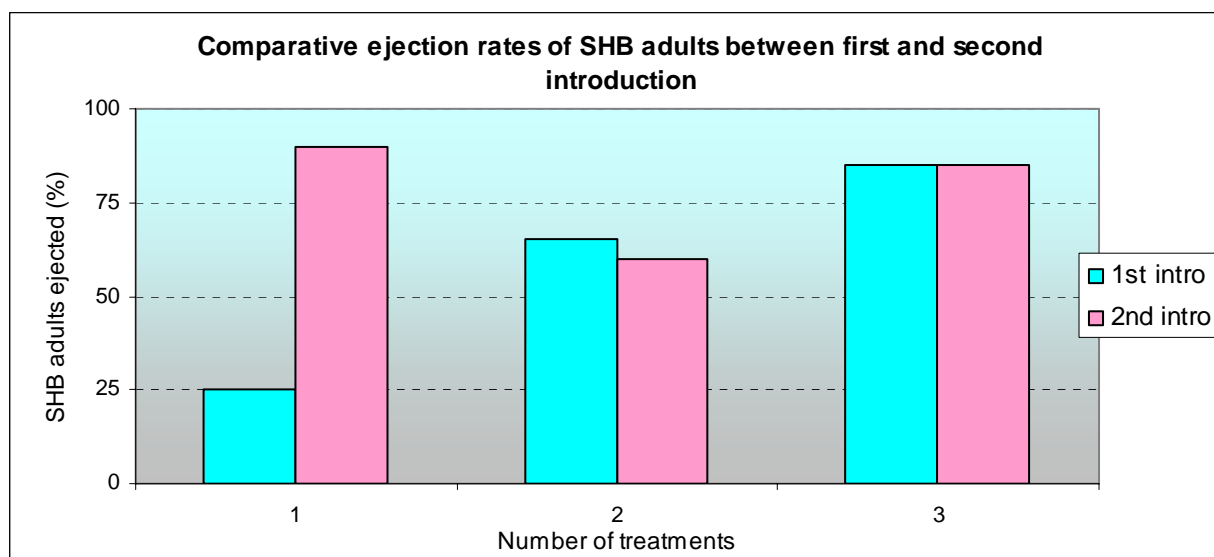
As a SHB adult was introduced to the entrance, guard bees grabbed at the beetle and hung on where they could. The tank-like shape of the SHB adult enabled it to push through the entrance defences, giving the guard bees no where to hold on as it passed them. With increased beetle introduction the number of guards visible at the entrance, and deeper into the tube, increased. At times guards were seen armed with resin (Figure 64).



**Figure 65: Bee riding SHB (left) with guards on standby**

There were several incidences where a bee was observed riding on the back of a SHB as it was chased out by numerous bees. Guard bees gathered near the rim of the entrance while one or two bees attacked the retreating SHB (Figure 65). Sometimes the bee would continue its attack by biting and wrestling until the SHB dropped to the ground; occasionally the bee still remained attached.

In the first week of this experiment initial ejection rate of introduced SHB adults by guard bees was as low as 25%. Over the next two release periods the ejection rate improved significantly, to 85% by the third treatment (Figure 66).



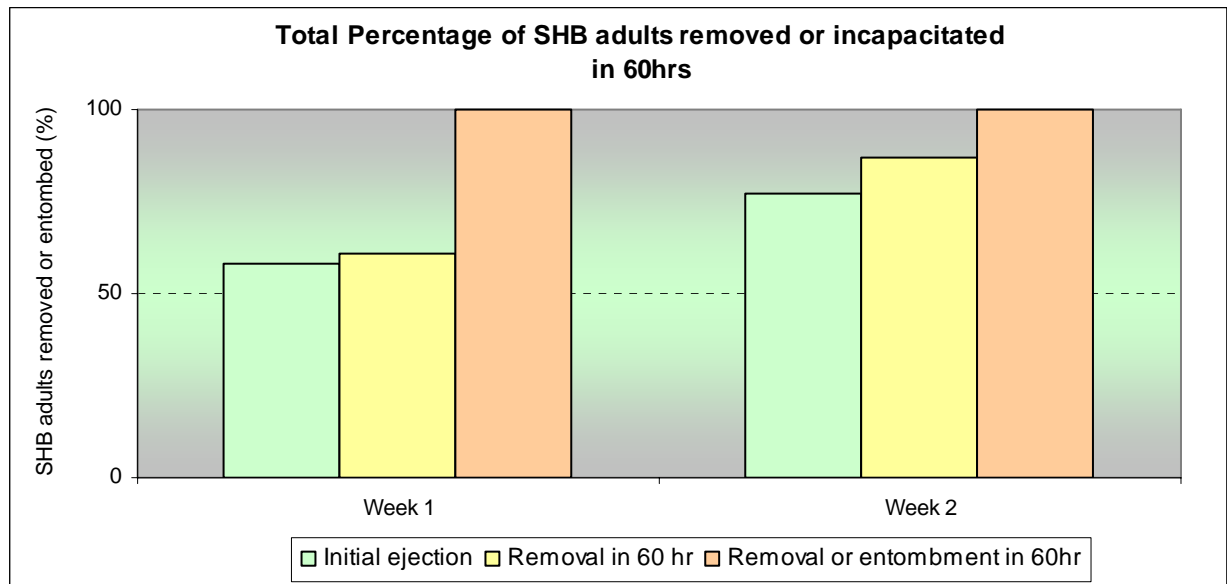
**Figure 66: Comparison between adult ejections on day one and day two**

These data were subsequently analysed by Dunnett's T3 test. The data approached normality after  $\ln(x+1)$  transformation. There was a significant interaction between the release attempts (1-3) within the day of the experiment, and the day the experiment was conducted ( $F_{2, 18} = 6.314$ ,  $p = 0.008$ ) so differences between attempts were analysed for each experimental date separately.

On November 2, 2006, there were significant differences between attempts in the number of SHB adults ejected ( $F_{2, 9} = 6.616$ ,  $p = 0.017$ ). Dunnett's T3 test shows that significantly more SHB adults were ejected in the third attempt than in the first attempt. There were no significant differences between the first and second attempt or between second and third attempt.

On November 28, 2006, there were no significant differences between attempts ( $F_{2, 9} = 2.167$ ,  $p = 0.170$ ) (See Appendix C for data analysis).

Finally, a total of 58% of the SHB adults were ejected during the first set of experiments and this increased to 77% in the second set of experiments (four weeks later). An additional 3% (for the first set of experiments) and 10% (the second set of experiments) of the introduced SHB adults were removed from the OP during the following 60 hours. The remaining SHB adults were entombed within the hive structure. A total of 100% of all SHB adults introduced into the hive entrances, were removed or entombed in experiments one and two (Figure 67).



**Figure 67: Total removal or destruction of adults**

## 6 Discussion

With the spread of the African SHB outside its native range (Ellis 2002a), the commonalities of hive contents, that of honey, pollen, wax and brood, between *Apis mellifera* and stingless bees, present a threat to the latter species as potential new hosts, because these components form a highly attractive food source for SHB. The migratory practices of Australian bee keepers aid the spread of the SHB, as they move the SHB-susceptible hosts in and out of native woodlands and forests around the country. Infested *Apis* colonies may be a potential source of SHB contamination for both feral *A. mellifera* and native bee colonies within national parks and other woodlands, as they can produce pupating SHB in soil to emerge and seek alternative food sources (Neumann et al. 2006). The stingless bee hives, with their colony contents, could potentially provide this alternative food source, in the absence of *A. mellifera* colonies.

If stingless bees such as *Austroplebeia australis* are susceptible to SHB invasion, wider ramifications may include the potential for stingless bee colonies, both managed and natural, to become reservoirs of SHB, for returning or newly introduced honeybee hives. This cycle could potentially devastate populations of social bees (Neumann and Elzen 2004) thus threatening the sustainability of native flora and fauna, as well as horticultural and agricultural crops.

Therefore, the investigation and subsequent improved understanding of *A. australis* colonies' vulnerability to SHB invasion is an important step in the development of management strategies for sustainable horticultural and agricultural practices, for the wide range of crops requiring insect pollination.

### 6.1 Comparative observations of *A. australis* colony behaviour in the absence and presence of SHB

One of the aims of this project was to develop methodologies by which the natural behaviour of *A. australis* could be observed and recorded, with minimum disturbance. I was able to develop suitable techniques, using the Observation Platform (OP) as an extended part of an OATH hive box, to observe the bees' "normal" in-hive behaviour. These observations essentially served as a "control" treatment in the absence of SHB, which was able to be compared with bee behaviour in the presence of SHB. Normal behaviour was performed in a busy but calm manner. On hot, sunny days hive activity increased, with more foragers exiting and returning to hives (Heard and Hendrikz 1993), but the general range of hive activities remained ordered.

The house-keeping activities and the deposition of faeces in specific areas within the hives and OPs observed throughout the experimental period have been described by previous researchers

(Rayment 1935; Michener 1974; Dollin 1996a) as normal in-hive activities. It was observed that extraneous inert material, such as cotton wool, paint brush bristles, faecal debris or latex shavings, did not stimulate the urgency or intensity of activity within the colony, as did introduction of SHB. It is therefore clear that the behaviour of the bees towards the introduced SHB was not one of hygienic behaviour, but of defence.

Both hive entrance and in-hive communication were observed throughout the experimental period, with trophallaxis and antennal contact being the most common (Michener 1974; Sommeijer et al. 1983; Kerr 1994; Nieh 2004). Exchanges observed in the absence of SHB were usually brief, from one to ten seconds, but with the introduction of SHB adults to hive entrances, the communication exchanges increased in both length (up to 25 seconds) and intensity. These lengthy communications may also assist the spread of alarm pheromones, thus stimulating guard recruitment at the hive entrance.

Wing fanning was observed from the beginning of spring (September). It has been reported that this behaviour is used to cool hives (Wille 1983; Dollin 1996a) and aid in gaseous exchange (Moritz and Crewe 1988) in stingless bees. Increased external temperatures coincided with increased incidences of wing fanning within the hives which was considered as normal hive cooling. However, increased wing fanning was also observed in the presence of SHB adults (Section 5.2.3). This may be a mechanism to assist the spread the alarm pheromone and therefore to recruit more guards.

Fine resin curtains have been reported to be constructed at the front entrance of *Austroplebeia* hives (Dollin 1996c), and these structures were occasionally observed when the hives were located externally in a garden. In this location, hives were directly exposed to night-time temperatures as low as 6°C. Under such conditions, bee flight activity the following day was limited to the late afternoon (13:00-15:00 EAST). In addition, in-hive activity was low during the mornings. The absence of resin curtains at the hive entrances, after the transfer of these hives to the CT rooms, may have resulted from the increased minimum temperatures (21°C) to which these hives were exposed. While it was expected that a key function of the resin curtains was to restrict entry of intruders, these results suggest they may also play another major function: that is to reduce cold air entry into the hive during cool nights and in winter. This may explain why curtain construction did not occur in these CT room hives

While in the external location the four experimental colonies had effectively “shut down” with the onset of winter, due to reduced foraging, thus ceasing active brood production, However,

following their relocation to the CT rooms and the provision of food supplements, the colonies “switched” to brood production, with the resumption of brood cell construction and POP activity (Drumond et al. 1999). This brood production throughout winter resulted in higher worker populations in the following months, ensuring rapid colony growth once optimal flying and foraging conditions occurred. Increased flight activity coincided with increases in external temperatures and day length and as flight activity increased, so too did the number of foragers returning with pollen (Section 5.1.3. In spring there were greatly increased pollen and nectar sources within the hives’ foraging range, including extensive stone fruit (*Prunus* spp) and citrus (*Citrus* spp.) plantings, ornamental pear *Pyrus calleryana*, native flora (*Eucalyptus* spp) and Cape weed (*Arctotheca calendula*). Although high numbers of other bee species, *A. mellifera* and *T. carbonaria* were also observed foraging within the same area, *A. australis* was able to forage competitively, enabling them to pack pollen, store honey and increase hive content weight.

Colony strength is of utmost importance in the ability of eusocial bees to defend themselves against intruders, especially against the SHB (Lundie 1940; Hepburn and Radloff 1998). As foraging conditions and flight activity were limited during the colder months (Figure 40) the *A. australis* colonies were supplied with supplementary honey and pollen (to supply carbohydrates and protein, respectively) to aid in brood rearing (Loper and Berdel 1980; Michener 2000). It is a common practice in Australia to provide overwintering *A. mellifera* colonies with sugar syrup and pollen supplements, and studies have shown a reduction in colony population of 78% where supplements were not provided, compared to a reduction of only 6% where supplementary feeding was provided (Dadant 1975). By comparison, under the supplementary regime reported in this thesis, the *A. australis* colonies recorded a mean hive content weight loss of only 17% over the four coldest months (May to August), but their mean weight increased by 90% during the first month of spring (29 August 2006 – 25 September 2006) (Figure 39), reflecting their ability to instantly recruit foragers once environmental conditions were suitable.

## 6.2 Responses of *A. australis* to introduction of SHB into colonies

Nest-mate fighting, which was first observed, when hives were initially opened after two days of transport, was also observed during the introduction of SHB adults, but, in this case, within the OPs. Intercolonial fighting swarms in Australia of *Trigona* (Dollin 1996a) and *Austroplebeia* (pers. comm.. M. Duncan, R. Gloag April 2006) as well as Brazilian resource rivals, *Trigona spinipes* and *Melipona rufiventris* (Nieh et al. 2004) have been previously reported. However, there have been no reports of within-colony nest-mate fighting. Michener

(1974) suggested that the glandular secretions used as foraging trail marker by *Trigona* may act as an alarm pheromone when produced in high concentrations, which could be the case within the sealed experimental hives or with high numbers of numbers in the small space of the OPs.

While the defensive behaviour of *A. australis* appears to be similar to that of *A. mellifera* against eggs and larvae of SHB, their respective behaviours against adult SHB adults are quite different. *A. mellifera* have difficulty picking up or stinging the adult beetle, due to its “turtling” defensive behaviour (Elzen et al. 2001; Neumann and Elzen 2004), and the beetle is also able to hide in cracks and crevices within hives because it is much smaller than *A. mellifera* workers (Neumann et al. 2001a). Failure to remove the beetles from the hive is followed by “social encapsulation”, of SHB adults within the hive through construction of propolis prisons and confinement (Neumann et al. 2001a; Ellis et al. 2003c). In the *A. australis* hives, bees confined the beetles, but they achieved complete entombment of them rather than just “social encapsulation”. This resulted in 100% mortality, and dismemberment of several of these entombed beetles within 60 hours of their incapacitation.

During each introduction of SHB, irrespective of life stages, the initial reaction from the bees upon discovering their presence, was to search for and seal “breeches” within the OP or hive box. This behaviour even occurred when adult SHB were placed at the front entrance of the hive and thus when breeches did not exist. It therefore appears that the location and sealing detected breeches within the nest structure is a major early activity in colony defence.

Of the known cases of SHB invasion of Australian stingless bee hives (all *T. carbonaria*), all had been either severely weakened by extreme temperatures ( $> 42^{\circ}\text{C}$ ) (pers. com. M. Duncan January 2006, A. Dollin April 2006), physically damaged through tree felling (pers. com. R. Luttrell April 2006; C. Alonso February 2007) or had been recently divided (pers. com A. Dollin April 2006). In the case of colony damage by high temperatures, bee populations are likely to be so low that an invasion by SHB could go unchallenged (Lundie 1940; Hepburn and Radloff 1998; Neumann and Elzen 2004). In the case of severe physical hive damage, bees may be unable to reseal large breeches quickly enough to exclude incoming adult SHB; infestations with the resultant larvae could cause considerable damage to hive contents, including brood. In cases where the hives have been successfully resealed soon after being damaged or split, colonies appear able to effectively remove both larvae and adults (pers. com. C. Alonso; R. Luttrell February 2007). There have been a number of reports where SHB are attracted to healthy stingless bee colonies and observed near hive entrances but no damage has resulted from their presence (pers. com. A. Dollin April 2006).

Compared to the reported behaviour of *A. mellifera* workers, when they encountered SHB eggs or larvae, the observed behaviour of the *A. australis* workers was not markedly different. In the studies reported in section 5.2, investigating the response of *A. australis* to different life stages of SHB, introduced SHB eggs were inspected in a calm and inquisitive manner, with approximately 50% of introduced eggs being consumed within 30 minutes. The consumption of SHB eggs by *Apis* has been reported (Spiewok and Neumann 2006a) while the consumption of worker eggs by the queen is common in *Plebeia remota* (van Benthem et al. 1995), *Melipona subnitida* (Koedam et al. 2005) and has been reported to occur only occasionally in *A. australis* and *A. symei* (Drumond et al. 1999). Consumption of worker eggs by fellow workers has also been reported (Koedam et al. 2007), and it could, therefore, be assumed that consumption of SHB eggs by workers in the experimental colonies could be a natural defensive behaviour.

Introduced SHB larvae were immediately challenged by the bees and activity within the OP immediately increased. Most larvae were quickly carried out of the entrance although some larvae were carried around in the OP for a short time prior to being removed. When bees worked independently larvae were removed more quickly and efficiently than by those working in pairs or groups. In some instances, bees were observed fighting over the possession of a larva, especially in one hive (Hive 3). There was a significant increase ( $P < 0.001$ ) in larval removal between treatments, showing an improved defensive behaviour with the second treatment. African honeybees, which are “resistant” to SHB, have also been reported to be efficient at the removal of SHB larvae, which they “jettison” up to 20 metres from the hive entrance (Neumann and Elzen 2004; Neumann and Härtel 2004). *A. australis* hive entrance activity was not able to be observed for the experimental hives during the introduction of SHB, so it was not possible to determine whether workers “jettisoned” the larvae or just dropped them within half a metre of the entrance, as they did with hive debris.

When the SHB adults were introduced they too were immediately challenged and the activity within the OP continued to increase and escalate as time passed from the moment of introduction. Bee behaviour became more energetic and long exchanges of trophallactic communication were observed. Worker numbers, within the OP, increased as more bees were recruited from the main hive. There were instances of nest-mate fighting and two cases of “balling”, as described in section 5.1.1, which could be attributed to increasing pheromone concentrations within the OP (Michener 1974).

Initially, the number and behaviour of the entrance guard bees were not sufficient to halt the advances of SHB adults at the hive entrance. Guard recruitment and subsequent increased

numbers resulted in a significant increase ( $P=0.017$ ) in ejection rates by the time of third introduction. It was interesting to note that during the second set of investigations four weeks later, there was no significant difference in colony response between any of the introductions, and that the colonies were better at ejecting adult beetles than when they were SHB-naïve. At times it was observed that the number of guards in attendance at the front entrance had increased from four to nine and there were bees along the length of the tube, from the outside to the OP. Colonies were also observed creating small stock-piles of soft resin within the OP and using these to supply ammunition against SHB.

The use of resinous substances such as cerumen and wax as an important defensive behaviour observed in *A. australis* ( in our case against adult SHB) has also been described against other predators by *Trigona*, *Plebeia* and *Tetragona* (Michener 1974; Dollin 1996a). While *Austroplebeia* colonies contain a lower volume of resin in comparison to *Trigona* (Dollin 1996c), the bees in the experimental hives were observed harvesting sufficient amounts of resin and cerumen from within the hive to incapacitate and entomb all introduced SHB adults.

When the OPs or the lids were substituted for new ones, during the experimental period, small resin stockpiles reappeared on the new lids within a few hours. Some researchers have reported that social insect colonies have evolved both communal and individual “immune responses” by developing defence strategies that deal with a previously encountered threat such as parasites and pathogens (Evans and Pettis 2005). In these situations, the colony, acting as a “super-organism” (Moritz and Southwick 1992), may behave like a macro-organisms and develop responses when exposed to antigens (Brunner and Suddarth 1975), to better equip them to defend against future attacks. However, this response, which appears to vary across colonies of *A. mellifera*, significantly reduces productivity as hive activity is “traded off” between colony defense and production (Evans and Pettis 2005).

### 6.3 Stingless bee management strategies to reduce SHB infestations

As the threat of the SHB spreads throughout Australia and the world, and based on the results described in this thesis, it is recommended that stingless bee nests be closed and sealed as soon as possible after they are damaged and that current meliponiculture management techniques be reviewed. For example, both hive splitting to propagate colonies and opening hives to harvest hive products reduce the colony’s defences against potential SHB invasion. Utilization of a closed, protected environment such as a room or shed for such hive manipulations could significantly decrease the possibility of SHB invasion into open hives.

## 6.4 Conclusion

Although the bees in this study were only partially successful in their attempts at SHB removal, they successfully destroyed or incapacitated those that remained within the nest, thus removing any threat to the colony. It should be noted that the normal mechanism for intact hive invasion by SHB would be by adult beetle entry through the hive entrance, *A. australis* demonstrated its capability to successfully defend its colonies in this scenario. Thus, healthy, strong colonies maintained in undamaged hives, whether wild or managed, appear to be able to defend against hive entrance attack by SHB. However, if a small number of SHB did successfully infiltrate the nest, the data suggest that *A. australis* would still successfully defend themselves through incapacitating or removing the intruders.

The long term implications of the presence of SHB in Australia and the world are complex. The threat to European honeybees by SHB, and the possible reduction in their populations, could potentially impact on the horticultural and agricultural industries due to declining insect pollinator availability. The inability of European honeybees to prevent hive invasion by SHB makes the importance and availability of alternative crop pollinators paramount. The defensive behaviour of *A. australis* against the SHB gives this native bee an advantage over its introduced *Apis* cousins. A better understanding of the behaviour of *A. australis*, especially towards hive invasion by SHB, may enable the sustainable exploitation of this remarkable bee species (Kakutani et al. 1993; Heard 1999).

## 6.5 Further suggested research

The experimental work was conducted within the grounds of the apiary at UWS. This ensured a ready supply of *A. mellifera* colonies for SHB to inhabit, within flight distance of the *A. australis* hives. At no time during the experimental period were SHB observed near *A. australis* hive entrances nor within the OPs or hive boxes, unless they were intentionally introduced as part of planned investigations. With this ready supply of their preferred food, namely living honeybee hive contents (Ellis 2002a; Ellis et al. 2002b), the SHB had no incentive to invade the hives of stingless bees. Further work to assess the attractiveness and vulnerability of *A. australis* to invasion by SHB, could introduce strong stingless bee colonies into areas recently vacated by *Apis* hives used for honey production. Adult SHB emerging from pupae in the soil would be likely to readily seek new food sources. In the absence of the original honeybee hives SHB may be more likely to naturally invade the stingless bee hives. These investigations would better replicate the potential scenario posed by bee keeper migration of *Apis mellifera* hives into National Parks and stands of native vegetation.

The experiments developed for the study of *A. australis* could also be applied to the study of other stingless bee species in Australia and elsewhere, to evaluate their vulnerability to SHB invasion.

The comparative ability of colonies to develop communal “immune response” to SHB and the subsequent trade-off against productivity could be further investigated using methods developed in my studies. In particular, guard recruitment and use of soft resin stock-piles could be evaluated.

The importance and role of pheromones, particularly alarm pheromone, in colony defense in *A. australis* and other stingless bees also requires further investigation.

Finally investigations of stingless bee hive temperatures and their relationship to external temperatures and hive “shut down” vs. brood production, as well as production of resin curtains, could be further studied as part of *A. australis*’ ability to defend their colonies against SHB invasion.

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## Appendix A

### Hours above 18°C (weekly)

Date	Min °C	Max °C	No: hrs
17-May	18.8	25.5	30
24-May	18.1	24.1	33
31-May	18.4	26.3	32
7-Jun	18.1	25.9	26
14-Jun	18.1	23.4	13
21-Jun	18.1	24.5	21
28-Jun	18.1	20.6	3
3-Jul	18.2	27.4	15
12-Jul	18.1	25.9	27
19-Jul	18.4	24.1	7
2-Aug	18.4	23.7	30
9-Aug	18.1	24.5	14
15-Aug	18.1	23.4	31
22-Aug	18.1	25.9	38
29-Aug	18.1	27.7	40
5-Sep	18.1	29.2	45
12-Sep	18.8	25.2	16
19-Sep	18.1	29.9	39
26-Sep	26.8	29.8	68
3-Oct	18.1	34.9	55
11-Oct	18.1	38.1	55
17-Oct	18.1	38.5	48
24-Oct	18.1	34.1	48
1-Nov	18.1	37.3	53
8-Nov	18.1	37.3	48
15-Nov	18.1	36.1	64
22-Nov	18.1	41.5	57
29-Nov	18.1	40.2	72
6-Dec	18.1	41.1	55
13-Dec	18.1	41.9	67

## Appendix B

### Correlations between flight activity and pollen collection

#### Descriptive Statistics

	Mean	Std. Deviation	N
flight	40.91	32.264	76
pollen	5.83	7.866	76

#### Correlations

		flight	pollen
flight	Pearson Correlation	1	.714(**)
	Sig. (2-tailed)		.000
	N	76	76
pollen	Pearson Correlation	.714(**)	1
	Sig. (2-tailed)	.000	
	N	76	76

\*\* Correlation is significant at the 0.01 level (2-tailed).

### Regression

#### Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.714(a)	.510	.503	5.544

a Predictors: (Constant), flight

#### ANOVA(b)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2365.942	1	2365.942	76.964	.000(a)
	Residual	2274.835	74	30.741		
	Total	4640.776	75			

a Predictors: (Constant), flight

b Dependent Variable: pollen

#### Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-1.292	1.031		-1.253	.214
	flight	.174	.020	.714	8.773	.000

a Dependent Variable: pollen

## Appendix C

### Data analysis of SHB adult ejection from the hive entrances

#### Tests of Between-Subjects Effects

Dependent Variable: Inejct

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	48.719	1	48.719	78.987	.071
Hypothesis Error	.617	1	.617(a)		
attempt	.698	2	.349	.677	.596
Hypothesis Error	1.031	2	.516(b)		
day	.617	1	.617	1.196	.388
Hypothesis Error	1.031	2	.516(b)		
attempt * day	1.031	2	.516	6.314	.008
Hypothesis Error	1.470	18	.082(c)		

a MS(day)

b MS(attempt \* day)

c MS(Error)

#### Tests of Between-Subjects Effects

Dependent Variable: Inejct

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.519(a)	2	.759	6.616	.017
Intercept	19.186	1	19.186	167.152	.000
attempt	1.519	2	.759	6.616	.017
Error	1.033	9	.115		
Total	21.738	12			
Corrected Total	2.552	11			

a R Squared = .595 (Adjusted R Squared = .505)

#### Multiple Comparisons

Dependent Variable: Inejct

Dunnnett T3

(I) attempt	(J) attempt	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-.5493	.28984	.303	-1.6601	.5615
	3.00	-.8605(*)	.11114	.003	-1.2687	-.4523
2.00	1.00	.5493	.28984	.303	-.5615	1.6601
	3.00	-.3112	.27534	.640	-1.4871	.8647
3.00	1.00	.8605(*)	.11114	.003	.4523	1.2687
	2.00	.3112	.27534	.640	-.8647	1.4871

Based on observed means.

\* The mean difference is significant at the .05 level.

### Tests of Between-Subjects Effects

Dependent Variable: Inejct

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.211(a)	2	.105	2.167	.170
Intercept	30.150	1	30.150	620.672	.000
attempt	.211	2	.105	2.167	.170
Error	.437	9	.049		
Total	30.797	12			
Corrected Total	.648	11			

a R Squared = .325 (Adjusted R Squared = .175)