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Research article

The ontogenic time and worker longevity in the Australian stingless bee, *Austroplebeia australis*

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Abstract

Little is known about the biology and life cycle of the Australian stingless bee, *Austroplebeia australis* (Friese). The ontogenic times for developing offspring, as well as the longevity of adults, drive the overall life cycle of a social colony. The developmental times for brood within stingless bee species which build cluster-type nests, such as *A. australis*, are as yet unreported. A technique was developed whereby 'donor' brood cells were separated from the main brood cluster and 'grafted' into hive annexes, allowing workers from within the colony to access the brood 'grafts' for hygiene and maintenance activities, whilst enabling observation of developing brood. The mean ontogenic time for *A. australis* workers, maintained at ~ 27°C, was 55 days, which is similar to that reported for other stingless bees. The maximum longevity of *A. australis* was determined by marking cohorts of worker bees within five colonies. Workers within all colonies demonstrated extended longevity, with an overall maximum longevity of 161 days, with the oldest bee living for 240 days. Extended longevity may result from evolutionary adaptations to the floral resource scarcity, which is regularly experienced in semi-arid, inland Australia; the natural habitat of *A. australis*.

Keywords: brood development, cluster-type brood, resource scarcity

Introduction

The greatest diversity of stingless bees (Hymenoptera: Apidae: Meliponini) is found in the Neotropics, with 412 (80% of all species) described species (Camargo and Pedro, 2012). Australia is home to 15 species within two genera, *Tetragonula* Moure (1961) and *Austroplebeia* Moure (1961). Most stingless bees construct multiple layers of regular, horizontal brood comb, containing cells or cocoons, in the centre of a nesting cavity (Michener, 1974; Wille, 1983). There are a few species, however, that construct irregular clusters of brood cells which are arranged to fit into the long, narrow, irregular cavities of small trees or large limbs (Michener, 1961; Wille, 1983). Species within the genus *Austroplebeia* construct irregular, cluster-type brood chambers.

Unlike *Apis mellifera* Linnaeus (Hymenoptera: Apidae), stingless bees do not progressively feed their young, but mass provision each brood cell with sufficient food to support larval development and seal it immediately after oviposition (Sakagami and Zucchi, 1963). Once a brood cell is provisioned, a single egg is oviposited on top of the larval food and the cell is immediately sealed (Wittmann et al., 1991; Drumond et al., 2000; Michener, 1974).

Workers of *Austroplebeia australis* (Friese) construct spherical brood cells arranged in simple, irregular clusters. Open cells face outwards from the leading edge of the cluster in random directions (Michener, 1961; Dollin, 2010a). This architecture presents a challenge to investigating the development of offspring within the brood cell cluster. As each layer of brood is produced, the next layer of cells is soon built directly on top, obscuring the developing cells from view. A few researchers have successfully studied brood development within the regular, horizontal brood comb of *Melipona* Illiger and *Trigona* Jurine species (Kerr, 1950; Salmah et al., 1987; Salmah et al., 1996; Moo-Valle et al., 2004); however, data on brood development within irregular cluster-building species have not been reported.

The ontogenic period in bees begins at oviposition and progresses through larval development and pupation to emergence as an immature adult (imago) (Winston, 1991). In social colonies, the time spent in ontogenesis

strongly affects population growth as well as worker replacement. This influences a colony's capacity to collect and store food resources, influencing its health, strength, and long-term survival. Brood development is also highly dependent upon incubation temperatures (Howe, 1967; Fukuda and Sakagami, 1968; Winston, 1991) and ontogenic times differ between species in similar environments (Michener, 2000).

The longevity of individuals within a colony is also important in understanding the overall life cycle and development of the colony as a 'super-organism' (Moritz and Southwick, 1992). Longevity is highly dependent upon nutritional input (Maurizio, 1950; Haydak, 1970), and the provision of parental care for juveniles within a social colony extends the lifespan of its individuals (Sakagami and Fukuda, 1968; Carey, 2001 a, b). The division of labour associated with the advancing age of colony members is known as age polyethism (Wilson, 1971; Michener, 1974; Hartfelder et al., 2006), in which workers move from low-risk, in-nest tasks to high-risk, defence and foraging tasks as they age. This results in low mortality rates during the early stages of life with increasing mortality rates later in life. Individuals live longer when high-risk tasks are postponed (O'Donnell and Jeanne, 1995). Once workers move to foraging tasks their physical and metabolic activity increases, thus increasing molecular damage and morphological decline (Cartar, 1992; Brys et al., 2007). Simply put, foragers 'wear out' more quickly than house bees. Sakagami and Fukuda (1968) demonstrated that as food resources increase in availability, dictated by seasonal change and floral abundance, the number of workers required for foraging tasks increases. This, in turn, leads to an increase in the overall mortality of individuals within the colony.

The aim of the current study was to determine the ontogenic time and the longevity of *A. australis* workers at controlled temperatures and to compare these to other bee species. It was necessary to develop a technique by which developing brood cells could be easily observed, whilst still being maintained by colony workers.

Materials and methods

Ontogenic time

In spring (September) 2008, eight queenright colonies of *A. australis*, housed in artificial hives, were prepared in the following way. Each hive was constructed of 20 mm thick cypress pine (*Callitris* sp.) wood, with dimensions 320 x 180 x 110 mm and capacity of 7 L.

To allow for ease of access and monitoring of the colonies, a 3 mm thick transparent acrylic lid was fitted and this was covered with the original timber lid to exclude light. All colonies were housed in controlled temperature rooms maintained at 27.6 ± 1.7ºC and darkness, at the University of Western Sydney, Richmond, New South Wales, Australia (33°36'S, 150°45'E). Each colony was provided with external, free foraging access via an 8 mm (internal diameter) silicone tube from the hive entrance through the wall of the building.

Each hive box had two holes 10 mm in diameter drilled into one side and an observation platform (henceforth, 'platform') was attached to each hole with 8 mm (ID) silicone tubing (Halcroft et al. 2008, Fig. 1). An additional platform was installed between the entrance tube and the hive box for further colony monitoring. The platforms were left untouched for at least one month to ensure they were accepted by the colonies, as evidenced by the fortification of annexes and construction of cerumen structures within (Halcroft et al., 2008).

Forty-six days later, the lid of each hive was removed and a wooden toothpick was placed on the leading edge of the brood cluster (Fig. 2a). The toothpick became a frame upon which the colony could build new cells each day. The lids were closed and the colony was left undisturbed for a further seven days. The hives were then reopened and the toothpicks, along with the attached 'donor' brood cells, were carefully removed from the brood clusters. The toothpicks and harvested cells were transferred (grafted) into one of the attached platforms (Fig. 2b) and the date of graft recorded. The process of toothpick placement, brood harvest and grafting was repeated several times over a six-week period, with a total of 16 groups of brood (replicates) being produced.

The cells within each graft were observed for stages of brood development and emergence. Observations were carried out weekly until pupal cocoons became evident. After this time, observations were carried out every second day. Estimates of ontogenic times were based on the assumption that the queen oviposited each day. Initial and final brood emergence was recorded. For each graft, brood development times were then calculated, based on the difference between the date immediately prior to cell harvest and the date of final emergence. Means were compared using Case Processing Summary, in SPSS 17 (IBM Company, Chicago, Illinois).

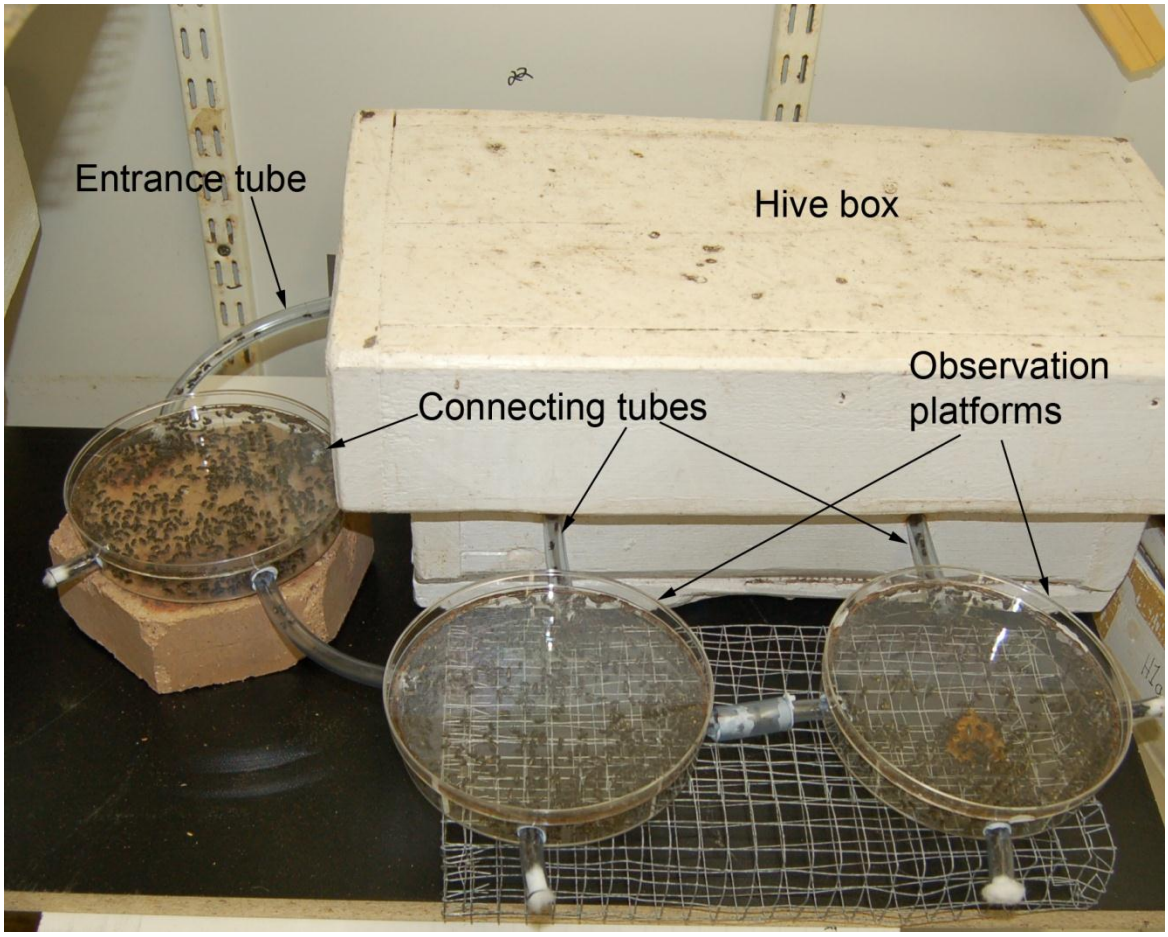

Fig. 1 Hive box containing the mother colony with observation platforms attached. It was possible to observe the grafted brood which was housed within the platforms. Colony workers were able to access the brood grafts via the tubing, thus caring for the developing young. The entrance tube enabled colonies to access the external environment.

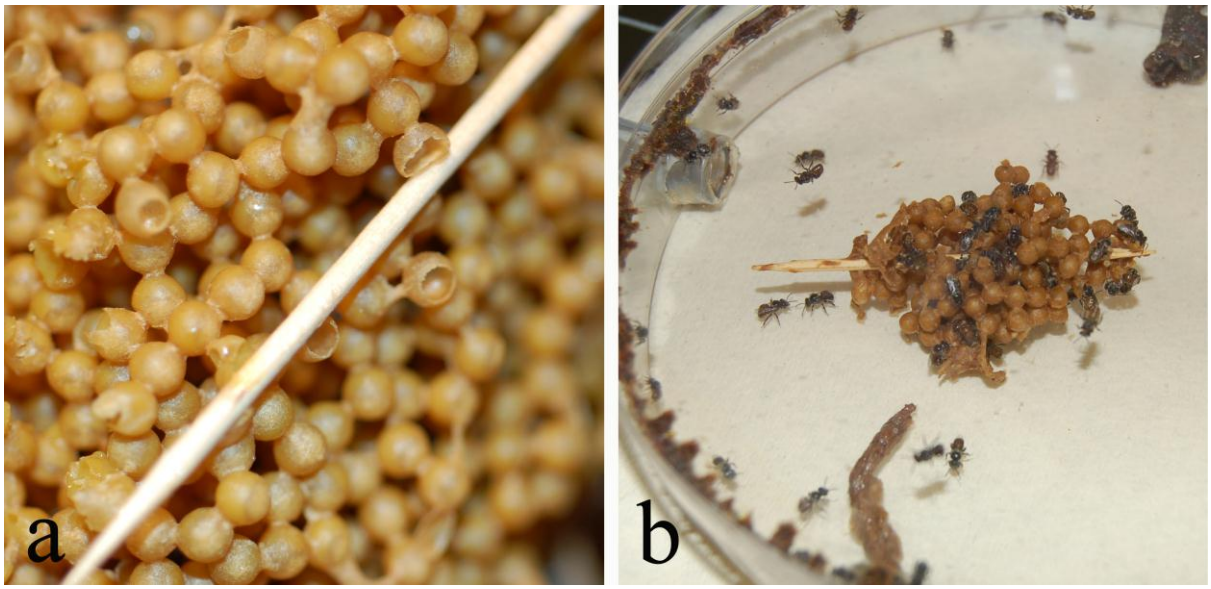

Fig. 2 a Toothpick positioned on the leading edge of the *Austroplebeia australis* brood cluster **b** Donor brood cells, attached to the toothpick, grafted into the observation platform

Longevity

It is difficult to mark and monitor a large number of imagoes within fully developed cluster-type colonies of *A. australis*, and marked bees are also difficult to locate within the nest structures. To overcome these problems, a section of pupating brood was removed from the mother colony and transferred to a small hive box (280 x 200 x 100 mm). Due to their small size (1.5 mm thorax), it was not possible to mark individual *A. australis* workers with a number, so individual workers were marked with acrylic paint of two different colour combinations.

On 25 May 2009 (late autumn), five *A. australis* colonies were prepared in the following way. A piece of pupating brood (~ 5 x 4 x 3 cm), along with an unknown number (but >50) of adult workers was separated from the brood cluster of a strong colony. The brood structure and its occupants were placed inside the hive box which was closed with a 3 mm thick transparent acrylic lid. Supplementary food sources were provided in the form of a mixture of water and *A. mellifera* honey (water: honey, 1:5 v/v) as well as a section of *A. australis* pollen pots (3 x 2 x 2 cm). Honey (20 mL) was replenished every two weeks. The colonies were maintained in controlled temperature rooms at 25.2 ± 0.03°C, monitored using 'Tinytag' temperature data loggers (Hastings Data Loggers, Port Macquarie NSW, 2444, Australia), and in darkness, at the University of Western Sydney, Richmond, New South Wales, Australia (33°36'S, 150°45'E). Hives were connected to an entrance tube which provided the colony with access to external foraging, as previously described. Ambient temperatures varied greatly during the study, from -2.4 – 26.8ºC in winter, 2.1 – 41.4ºC in spring, and 9.7 – 42.1ºC in summer. Rainfall in 2009 was low, with only 578 mm (72% of the long term average (BOM 2012)) and was, therefore, not a limiting factor for foraging activity.

A preliminary study showed that imagoes were lighter in colour than older workers, for up to six days after emergence. Imagoes were therefore marked every three days, to allow a greater number of bees to emerge and be marked for each cohort. As a result, the maximum difference in age of individuals within each cohort was three days. To mark the imagoes, a filter paper-lined Petri dish (90 mm) was placed in a shallow ice slurry. All visible imagoes were gently aspirated into a pooter. Collected bees were placed on the pre-chilled Petri dish, where it took less than five seconds for individuals to cease walking. Bees were chilled only to the point of cessation of movement and then the Petri dish was removed from the ice. Marking was carried out on batches of six to ten bees at a time to reduce the severity and duration of chilling trauma. Using acrylic art pens (Mitsubishi Pencil Co Ltd, Japan), two colours were placed on either side of the thorax; the marked bees were then warmed under a halogen light and returned to the hive. After 19 days, the five hives collectively contained 602 marked bees from 39 three-day cohorts (replicates).

As the colonies had access to the external environment it was not possible to collect dead workers. The colonies were examined weekly via the acrylic lid and workers' colour markings were recorded. When individuals bearing a particular combination of colour marking (e.g. red / white) were no longer observed in the hive, this date was recorded as the maximum age reached for members of that cohort (maximum longevity).

During the period of brood emergence and marking, a queen cell was observed in three of the five colonies. One of these colonies successfully re-queened during the study, thus producing one queenright colony and four queenless colonies. All colonies were observed to forage on warm (>20ºC), fine days, collecting and storing nectar and pollen, although some colonies were more active than others. The experiment was terminated 249 days after its commencement, when no marked workers remained in any of the hives.

The maximum longevity for each of the cohorts, within each hive, was recorded and the mean of these was calculated. Data were tested for equality of variance using Levene's test. The data were analysed by one-way ANOVA and compared by multiple comparison and Tukey's HSD test in SPSS 17 (IBM Company, Chicago, Illinois). The setting of significance was $\alpha = 0.05$.

Results

Ontogenic time

The platforms with 'grafted' brood were accepted by the colonies, even when the brood did not originate from that colony, and workers used cerumen to secure the brood cells to the floor of the platforms within hours of their graft. The mean ontogenic period of *A. australis* worker brood, at an average temperature of 27.6ºC, was 54.8 ± 1.1 days (range 49 – 63 days).

Longevity

The maximum longevity of the cohorts (n = 39) was 161.4 ± 6.1 days, ranging from 100 to 240 days. There was no correlation between the date of emergence and longevity. There was no significant difference ($p = 0.312$) between the maximum longevity of the queenless, broodless colonies (n = 31) (161.7 ± 7.4 days) and the queen-right, brood producing colony (n = 8) (160.5 ± 8.4 days).

During the first 85 days of the study, workers within the four queenless colonies produced between five and 38

drone cells. Of these cells, 43% contained viable drones. Drone production ceased after this time (mid winter). At the conclusion of the study, the colonies contained between 10 and 100 pollen pots and two to six provisioned honey pots. The queen-right colony had produced a brood cluster ~ 70 mm in diameter and had good stores of honey and pollen.

Discussion

The ontogenic period for *A. australis* workers (55 days at 28ºC) is 2.6 times longer than that of *A. mellifera* (21 days) (Jay, 1963; Winston, 1991). The extended development times of *A. australis* and a small number of previously studied stingless bee species, may be attributable to lower incubation temperatures. *Apis mellifera* actively incubates its brood between 30 and 35ºC (Winston, 1991). If incubation temperatures are allowed to move outside this narrow range, development times and survival rates are adversely affected (Himmer, 1927; Jay, 1959 cited in Mardan and Kevan, 2002).

The greatest diversity and abundance of stingless bee species is found in warmer climates (mean temperatures range 18 – 35ºC) between the Tropics of Cancer (23.5ºN) and Capricorn (23.5ºS) (Kerr and Maule, 1964). *Melipona beecheii* Bennet has an ontogenic period of 53 days, when incubated at ~30°C (Moo-Valle et al., 2004), and two Sumatran stingless bee species, *Trigona* (*Trigonella*) *moorei* Schwarz and *Trigona* (*Heterotrigona*) *itama* Cockerell, both have an ontogenic period of 46.5 days (Salmah et al., 1987; Salmah et al., 1996). While brood incubation temperatures were not stated for the Sumatran studies, the average ambient temperatures experienced within this area range between 23 and 31ºC (Climatemps 2012). Many stingless bee species do not thermoregulate their nests, and brood temperatures fluctuate with ambient temperatures (Wille, 1983; Roubik, 1989), possibly as low as 23ºC for these Sumatran species. It is speculated that the optimum incubation temperature for stingless bee brood is 30 – 32ºC (Engels et al., 1995) and some tropical species can maintain their brood chambers 2 – 4ºC either side of this optimum (Sakagami, 1982; Roubik and Peralta, 1983; Engels et al., 1995; Moo-Valle et al., 2000). These fluctuations may lead to seasonal variations in the ontogenic times for stingless bee species whose brood development can occur outside the narrow optimum temperature range.

Austroplebeia australis occurs in subtropical and warm temperate regions of Australia, such as south-east Queensland (Michener, 1961; Dollin, 2010b) and ambient temperatures in these regions range from -4 to 43ºC. *Austroplebeia australis* produces brood continuously, even during winter, but it does not thermoregulate its nest. Brood temperatures closely track ambient temperatures, especially below 15ºC (Halcroft 2012), which could lead to lengthened ontogenic times. This is likely to have an impact on colony strength, especially at the end of winter when colony food stores may be low and foraging activity resumes. Further investigations into the impact of incubation temperatures on stingless bee brood development are warranted.

The longevity of *A. australis* workers is substantially greater than that of any other reported highly eusocial bee species (Table 1), with the exception of winter honey bees (Sakagami and Fukuda, 1968). It could be anticipated that workers within broodless colonies would naturally have greater longevity, as they are likely to assume the physiological characteristics of winter bees (Maurizio, 1950). In honey bees, the phenomenon of long-lived or diutinus bees occurs when workers stop rearing brood and accumulate vitellogenin instead of converting it into brood food (Amdam and Omholt, 2002; Omholt and Amdam, 2004). In contrast, our study showed that the longevity of *A. australis* workers in brood-rearing and broodless colonies was similar, suggesting that there is no role for a vitellogenin-like compound in the longevity of *A. australis*.

The native range of *Austroplebeia* is mainly in inland arid and semi-arid areas of northern Australia (A. Dollin, pers. comm., 2009; Halcroft, 2012), which experience extended periods of drought. Colonies dramatically reduce foraging activity when floral resources are depleted. Unlike tropical bees, which are forced to remain within the nest during much of the wet season, *A. australis* workers choose not to leave, even when the weather is conducive to flight activity. An occasional scout bee can be observed leaving the nest during periods of floral dearth, but if resources remain scarce, foragers are not recruited and do not leave the nest (A. Beil, pers. comm., 2009; M.H., pers. obs.). O'Donnell and Jeanne (1995) showed that patterns of senescence in workers of social insect communities can be profoundly affected by delaying the performance of high-risk tasks. It would also lower the overall energy expenditure of workers who have become unemployed but are potential foragers. Workers, thus, remain within the safe confines of the nest, experiencing only low levels of molecular damage (Cartar, 1992; Brys et al., 2007). Such behaviour ensures that a proportion of the colony is still alive when food resources become available again. Once floral resources become abundant, scout bees recruit large numbers of foragers (A. Beil, pers. comm., 2009; M.H., pers. obs.)

Table 1 Maximum longevity (in days) of previously studied highly eusocial bee species

Species	Maximum	Source
***Austroplebeia australis* (Friese)**	**161**	**Current study**
Apis mellifera Linnaeus (spring / summer bees)	70	Sakagami and Fukuda, 1968
Apis mellifera Linnaeus (winter bees)	270	Sakagami and Fukuda, 1968
Melipona beecheii Bennet	101	Biesmeijer and Tóth, 1998
Melipona bicolor bicolor Lepeletier	68	Bego, 1983
Melipona compressipes fasciculata Smith	80	Giannini, 1997
Melipona favosa Fabricius	68	Sommeijer, 1984
Nannotrigona (*Scaptotrigona*) *postica* Latreille	60	Simões and Bego, 1991
Plebeia droryana Friese	75	Terada et al., 1975
Plebeia remota (Holmberg)	90	van Benthem et al., 1995
Tetragonisca angustula angustula Latreille	60	Grosso and Bego, 2002

Floral resources were adequate for foraging activity in Richmond during this study. Foragers were observed, although not monitored, entering and exiting all of the hives on warm, fine days. Compared to another Australian stingless bee, *Tetragonula carbonaria*, *A. australis* recruits relatively small numbers of foragers. These foragers do, however, work floral resources efficiently and with minimal energy expenditure (Halcroft, 2012). If, indeed, age-at-death correlates with the age at which workers take up high-risk, high-energy tasks, it is possible that the extended longevity of cohorts within the *A. australis* colonies is due to the fact that some workers never leave the nest. This phenomenon has been reported in other social bees (Biesmeijer and Tóth, 1998; Page and Peng, 2001). There is no evidence that the extended longevity of workers within *A. australis* colonies is due to physiological changes, such as vitellogenin accumulation, but may be an inherently behavioural trait utilised even under conditions where floral resources are accessible.

These studies indicate that the life cycle of *A. australis* workers, from egg to death, could feasibly extend to seven months or more. This provides the colony, within its harsh native environment, the opportunity to benefit from available, albeit unreliable, food resources, spanning all seasons.

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