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Research article

The thermal environment of nests of the Australian stingless bee, *Austroplebeia australis*

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Running headline – Halcroft et al. Thermal environment of *A. australis* nests

Abstract

The greatest diversity of stingless bee species is found in warm tropical regions, where brood thermoregulation is unnecessary for survival. Although *Austroplebeia australis* (Friese) naturally occurs in northern regions of Australia, some populations experience extreme temperature ranges, including sub-zero temperatures. In this study, the temperature was monitored in *A. australis* colonies' brood chamber ($n = 6$) and the hive cavity ($n = 3$), over a 12 month period. The *A. australis* colonies demonstrated some degree of thermoconformity, i.e. brood temperature although higher correlated with cavity temperature, and were able to warm the brood chamber throughout the year. Brood production continued throughout the cold season and developing offspring survived and emerged, even after exposure to very low (-0.4°C) and high (37.6°C) temperatures. *Austroplebeia australis*, thus, demonstrated a remarkable ability to survive temperature extremes, which has not been seen in other stingless bee species.

Key words: *cluster-type brood, thermoregulate, passive warming, metabolic heat*

Introduction

In highly eusocial insects such as stingless bees, the stabilisation of nest temperatures facilitates brood incubation and continuous development throughout the year, giving them a competitive advantage over many diapausal solitary species. Nest thermoregulation by social insects has been widely studied and colonies use two types of mechanisms, to maintain brood temperatures (reviewed by Jones and Oldroyd, 2006). Passive mechanisms include nest orientation and architecture to provide a buffering effect against environmental extremes. Active mechanisms refer to activities performed by individuals within a colony to manipulate the nest temperature. These activities include wing fanning and

evaporative chilling to cool the nest or activation of thoracic flight muscles and increasing metabolism to generate heat (Heinrich, 1974; Heinrich and Esch, 1994; Jones and Oldroyd, 2006; Macías-Macías et al., 2011).

Some species, such as *Trigona spinipes* Fabricius and *Scaptotrigona postica* Latreille, have been reported to incubate the brood chamber at similar temperatures to that of *Apis mellifera* Linnaeus (35°C) (Sakagami, 1982; Engels et al., 1995). Optimal temperatures for brood incubation in most stingless bees are considered to be between 28 and 36°C (Nieh and Sánchez, 2005) and many species are reported to maintain their brood chamber within this range (Roubik and Peralta, 1983; Engels et al., 1995; Moo-Valle et al., 2000; Nieh and

Sánchez, 2005; Torres et al., 2007; Sung et al., 2008).

The greatest diversity of stingless bees occurs in tropical regions (Sakagami, 1982), where average annual temperatures range from 20 to 30°C (Ritter, 2011), making thermoregulation of the colony unnecessary for survival. A small number of species inhabit the tropical highlands and are exposed to contrasting climates; e.g., *Melipona colimana* Ayala, which exhibits both cooling and heat-generating behaviours in response to temperature changes (Macías-Macías et al., 2011) and *Plebeia remota* Holmberg, which ceases brood production prior to the cold season (van Benthem, 1985). However, most Meliponinae do not actively generate heat and are, therefore, susceptible to local climate fluctuations (Roubik, 1989).

Stingless bees generally establish their nests within large cavities of living trees (Roubik, 1983; Roubik, 1989; Eltz et al., 2003; Samejima et al., 2004; Sung et al., 2008), which provide considerable insulation against ambient temperature extremes (Greves, 1964). Nests are constructed using a mixture of bees' wax and plant resin (cerumen). Batumen sheeting, an insulative layer of cerumen containing a higher proportion of resin and which may incorporate mud or plant matter, lines the tree cavity. The brood comb is constructed centrally and contains hundreds to thousands of developing offspring. The final nest modification is the construction of thin (<1 mm), laminal layers of cerumen, called involucrum. Some stingless bee species construct multiple lamellae which cover the entire surface of the brood comb (Chinh et al., 2005). These combined nest modifications enhance the intranidal microclimate, thus retaining heat produced by developing offspring (Roubik and Peralta, 1983; Roubik, 1989; Michener, 2000).

In contrast to many comb-building species, *Austroplebeia australis* (Friese) builds

cluster-type brood structures which conform to long, narrow tree cavities (Michener, 1961; Halcroft, 2012). This architecture creates a relatively large surface area within the brood chamber. *Austroplebeia australis* also constructs spherical brood cells with bee-spaces between the cells, allowing nursery workers and the queen access to the brood, thus exposing the surface of individual cells (Fig. 1). During the cooler months, a single involucral sheet is constructed over the surface of the brood cluster, encapsulating the brood and reducing the exposed surface area. In artificial hives, where the nest structure is less constrained by the cavity walls, honey pots are built over the leading edge of the brood, possibly providing additional insulation (Fig. 2 a, b). *Austroplebeia australis* prefers to inhabit long, narrow cavities within dead trees (Halcroft, 2012) which provide only minimal protection against temperature extremes. Similarly, minimal thermal protection is provided to colonies which are managed in man-made, wooden hives.

The native range of *A. australis* extends more than 2,000 km, from the districts of Fitzroy and Central West Queensland (23°31'S), through the Darling Downs and into northern New South Wales (31°04'S). The climate in its most southerly range is vastly different to the regions of central Queensland and is considered to be marginal for colony survival (Halcroft, 2012). The Darling Downs districts frequently experience very cold winters (e.g., -7.7°C (lowest recorded minimum) in Warwick, Queensland; 28°22'S, 152°03'E, elevation 475 m) and hot, dry summers (e.g., 42.7°C (highest recorded maximum) in Miles, Queensland; 27°18'S, 151°26'E, elevation 344 m) (Australian Bureau of Meteorology, 2012).

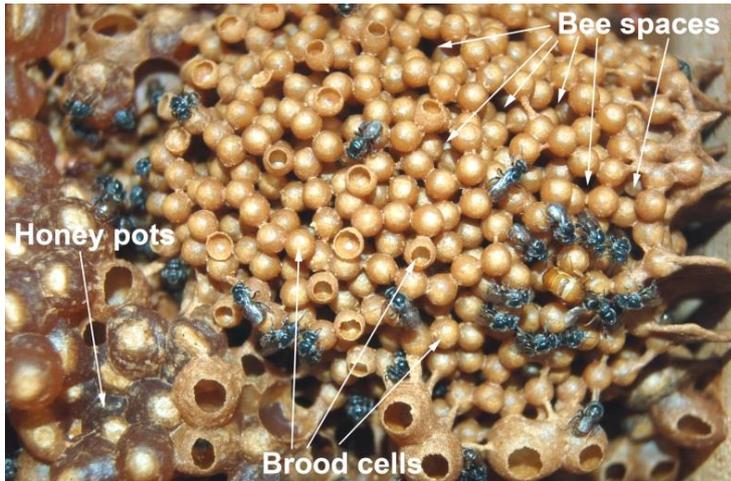
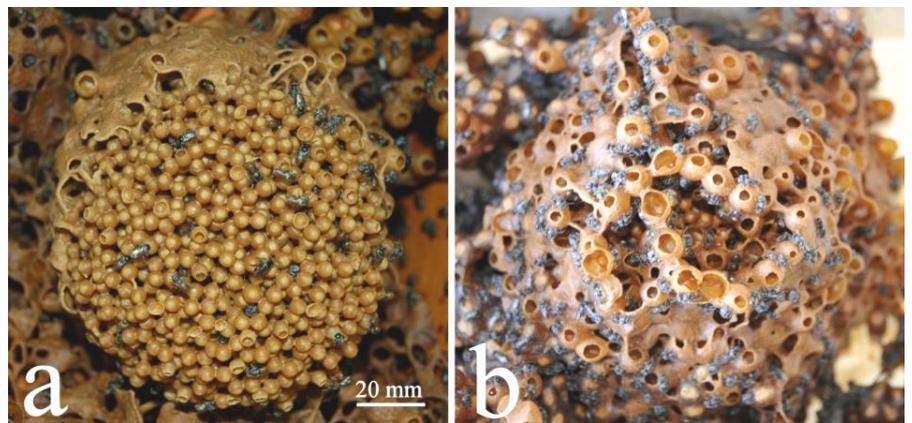


Figure 1 Cluster-type brood, constructed by *Austroplebeia australis* colonies. Irregularly arranged brood cells accommodate bee movement by providing bee-spaces between cells, which also increase the surface area of the brood assemblage. Bees were observed to occupy these bee-spaces at night and during the days when it was cold. This colony was housed within a hive box, where brood expansion was not constrained by the normally narrow walls of a natural tree cavity

Figure 2 a) Involucral layer, constructed by *Austroplebeia australis* workers, covering 10 – 20% of the brood cluster during summer (February) and b) a layer of involucrum plus honey pots constructed over 90 – 100% of the brood cluster during autumn (April). The brood chamber appears to occupy the same volume; however, the layer of honey pots conceals a much smaller brood cluster in autumn than the actively developing, exposed cluster seen in summer (a)



In this study we investigated whether *A. australis* colonies regulate temperature within the brood chamber. We observed brood production and development over a year, to examine their regulatory response, if any, for seasonal variations in temperature, including cold (<12°C) temperatures.

Materials and methods

On 21 October 2009, at Tara, Queensland (27°16'S, 150°27'E, elevation 314 m), six colonies of *A. australis* were transferred from tree hollows into individual hives constructed of 20 mm thick cypress pine, 320 mm x 180 mm x 110 mm, with a capacity of 7 L. Clear acrylic lids were fitted to the top of each box, to allow non-invasive observation of colony activities. A 20 mm thick cypress pine lid, insulated with 10 mm thick polystyrene sheeting,

was fitted over the top of the observation lid. The single entrance hole measured 10 mm in diameter. The six hives were transported ~900 km to Blaxland, New South Wales (33°45'S, 150°36'E, elevation 234 m), a location well south of the natural range of *A. australis*, and positioned on mesh shelving, underneath a north-facing, domestic decking, between 0.5 and 1 m above the ground. The northerly aspect of the decking provided the hives with full shade in spring / summer and sun in autumn / winter.

On 28 November 2009, a 4 mm diameter hole was drilled into the side of each of the hives and a 100 mm long x 2 mm diameter 'Fast response thermistor probe' (Hastings Data Loggers, Port Macquarie NSW, Australia) was carefully passed through the hole and into the brood cluster, causing minimal damage to the brood structure.

The tip of the probe was positioned to sit in the middle of the brood mass and secured into place and the probe was connected to a Tinytag Plus 2 data logger (TGP-4020, Hastings Data Loggers, Port Macquarie, NSW). The position of the probe was checked each month, to ensure it remained within the brood cluster throughout the study period. Three of the hives were also fitted with a 50 mm long temperature probe connected to a Tinytag Ultra 2 (Hastings Data Loggers, TGU-4020). The tips of these probes were positioned within the hive cavity, but not within any nest structures. This was to provide a comparative hive cavity temperature vs. brood temperature. A single Tinytag data logger was hung from the mesh shelving in the shade, to record ambient temperatures. Temperature data loggers were set to record at 30 minute intervals from December 2009 until December 2010.

Queenright status and active brood production were confirmed for each colony prior to commencement of the study. Assessment of the colonies was carried out on fine, sunny days (when possible) at the end of each month. During the coldest months, it was not always possible to see the leading edge of the brood to confirm active brood production, due to construction of involucre and honey pots over the brood chamber (Fig. 2b). The involucre was not disturbed during observations.

At the commencement of the study, and at the end of each subsequent month, the diameter of the brood cluster was measured. Measurements were taken using a metal ruler, which was inserted into the hive only when there was no risk of damaging the brood structures. Otherwise, the ruler was used as a guide only. The percentage of involucre coverage over the brood was estimated visually. Colonies were also checked for the presence of developing brood as well as newly

emerged bees (callows), which were lighter in colour for the first six days.

Laboratory observations on worker activity

To determine the temperature at which bees entered the stages of quiescence or chill coma, six replicate Petri dishes were prepared in the following way. To provide air flow, a 20 mm diameter hole was cut in the lid of the dish and a fine nylon mesh was glued in place. The dish was lined with filter paper and 10 mature, adult *A. australis* workers were transferred from one of six hives to each Petri dish. A refrigerated water bath (Thermoline Scientific, Wetherill Park, Australia) was fitted with a Unistat II heated circulator (Thermoline Scientific). The unit was filled with water and a small plastic bucket (~2 L capacity) was placed inside, and immersed to a depth of 100 mm, with the bucket lip ~50 mm above the water level. A thermometer probe (Jaycar Electronics, Rydalmere, Australia) was secured to the inside of the bucket and a Petri dish, supplied with 10 workers, was placed in the bucket. A Perspex lid was attached to the bucket to facilitate observation. The system was set at 20°C and cooled to 5°C, which took ~1 h. Throughout this period bee activity was observed as the temperature within the Petri dish decreased.

Statistical analysis of daily mean temperatures was conducted using general linear models repeated measures ANOVA in SPSS 21 software (IBM Corporation, Armonk, NY, USA).

Results

Unfortunately, the ‘ambient temperature’ data logger malfunctioned on 13 January 2010 (16 days into the study), and this did not become apparent until the conclusion of the study. Subsequently, hourly weather data were obtained directly from the closest automated Bureau of Meteorology

station at Penrith Lakes (station number 67113) (Australian Bureau of Meteorology, 2010), ~8 km north-east of Blaxland, but at an elevation of only 25 m, 200 m lower. These data showed that summer temperatures ranged from 12.3 to 42.6°C, averaging 23.7°C, and winter temperatures ranged from -0.6 to 24.2°C, averaging 11.5°C. In the absence of site-specific ambient temperature data, comparisons were made between the cavity and brood temperatures.

For the 16 days that ambient temperature ($n = 767$) was logged at Blaxland, in mid-summer, there was no evidence of nest cooling. Ambient temperatures ranged from 16.7 to 33.7°C (averaging 22.2°C ±

0.13) and the brood temperature was consistently higher than ambient (Fig. 3). On average, the brood temperature remained 4.9°C ± 0.08 higher than ambient for 96% of the time.

During this 12 month study, the *A. australis* colonies did not maintain a constant temperature within the brood chamber ($n = 6$), and temperatures ranged from -0.4 to 37.6°C. The brood temperatures correlated with the temperatures within the hive cavity ($n = 3$) which ranged from -1.4 to 37.9°C, maximum, mean and minimum temperatures are presented in Fig. 4 a, b and c, respectively.

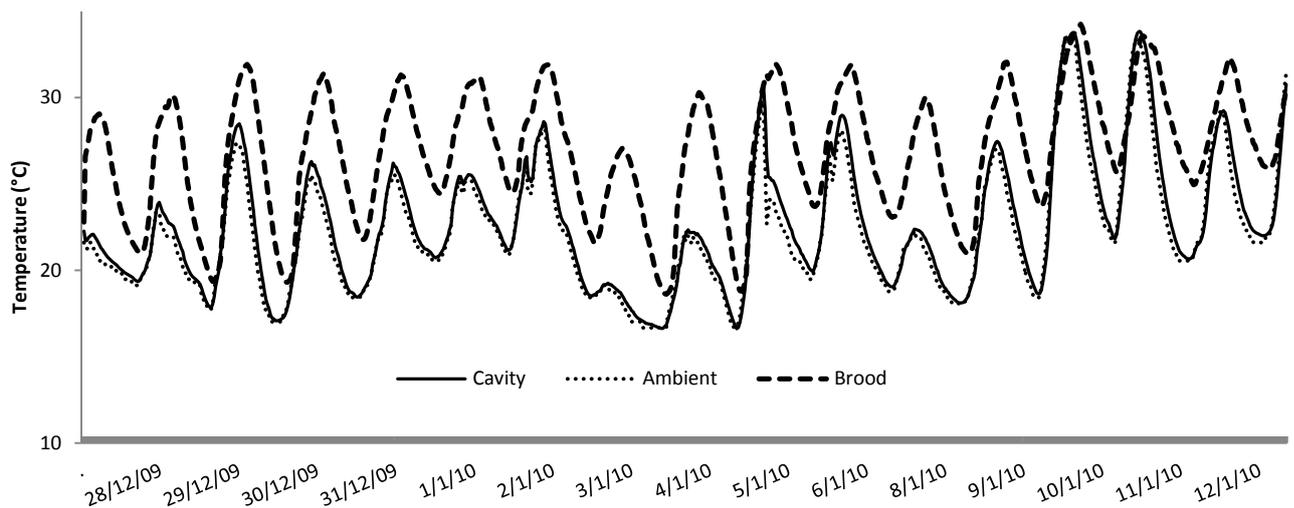


Figure 3 Temperature data logged at Blaxland, NSW during the first 16 days of the study (summer). Brood temperature was consistently higher than both cavity and ambient temperatures, indicating that the *Austroplebeia australis* colonies did not cool their nests

Colonies exhibited diurnal temperature fluctuations, and daily patterns saw the cavity temperature fall to its minimum about one hour after sunrise, with the brood falling to its minimum 1 – 2 h later (Fig. 5). Cavity temperatures peaked between 12:30 and 14:30 h, in the cooler seasons (Fig. 5b), and between 15:00 and 16:30 h during spring and summer (Fig. 5a, c). Brood temperatures rose from their minimum during the morning and continued to rise until the end of the day,

when cavity temperatures were falling (Fig. 5). Brood temperature peaked toward the end of the foraging day, up to 5 h after the cavity reached its maximum temperature (Fig. 5).

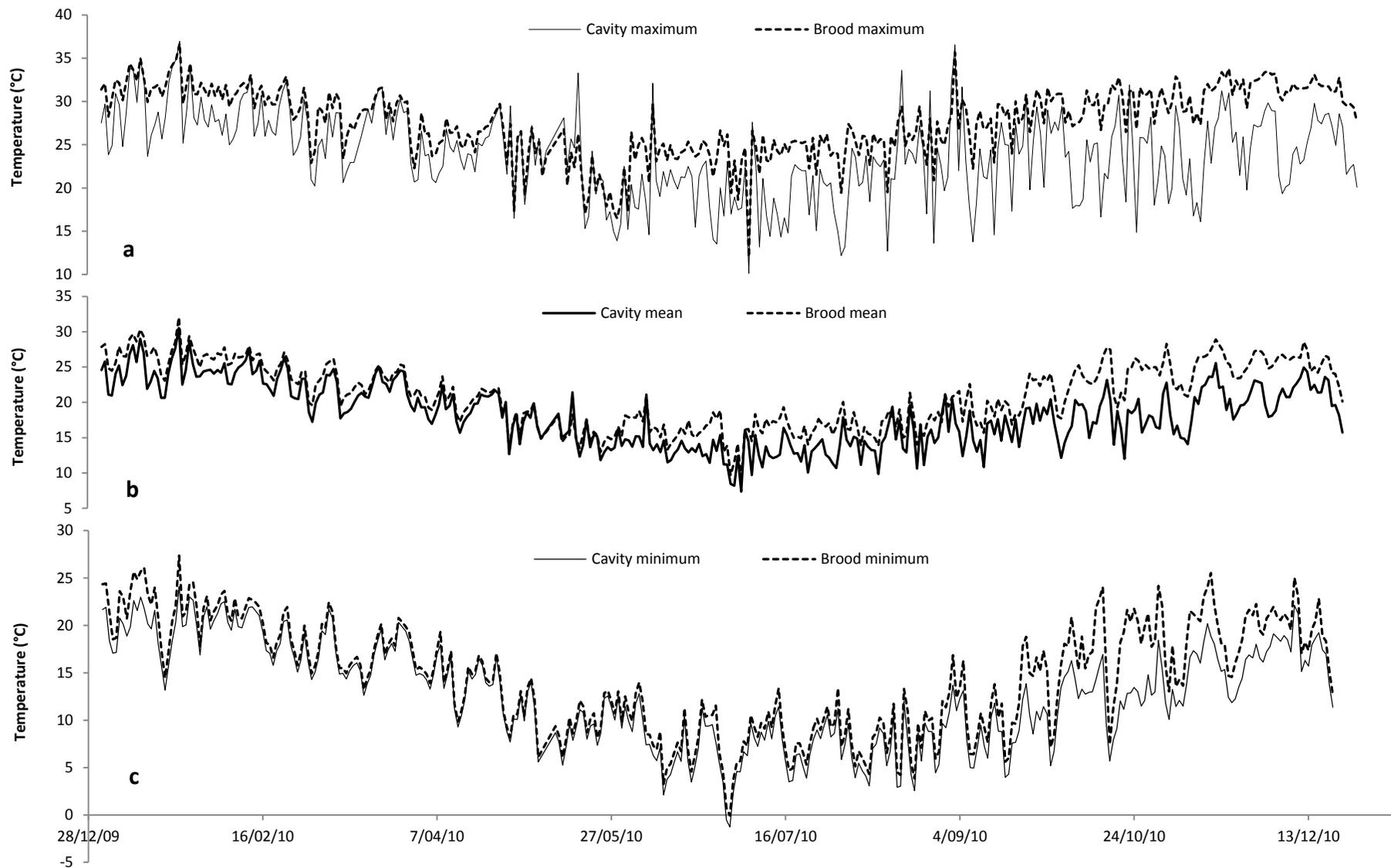


Figure 4 Temperatures based on the daily maximum (a), mean (b) and minimum (c) for the *Austroplebeia australis* brood cluster (n = 6) and hive cavity (n = 3) over 12 months

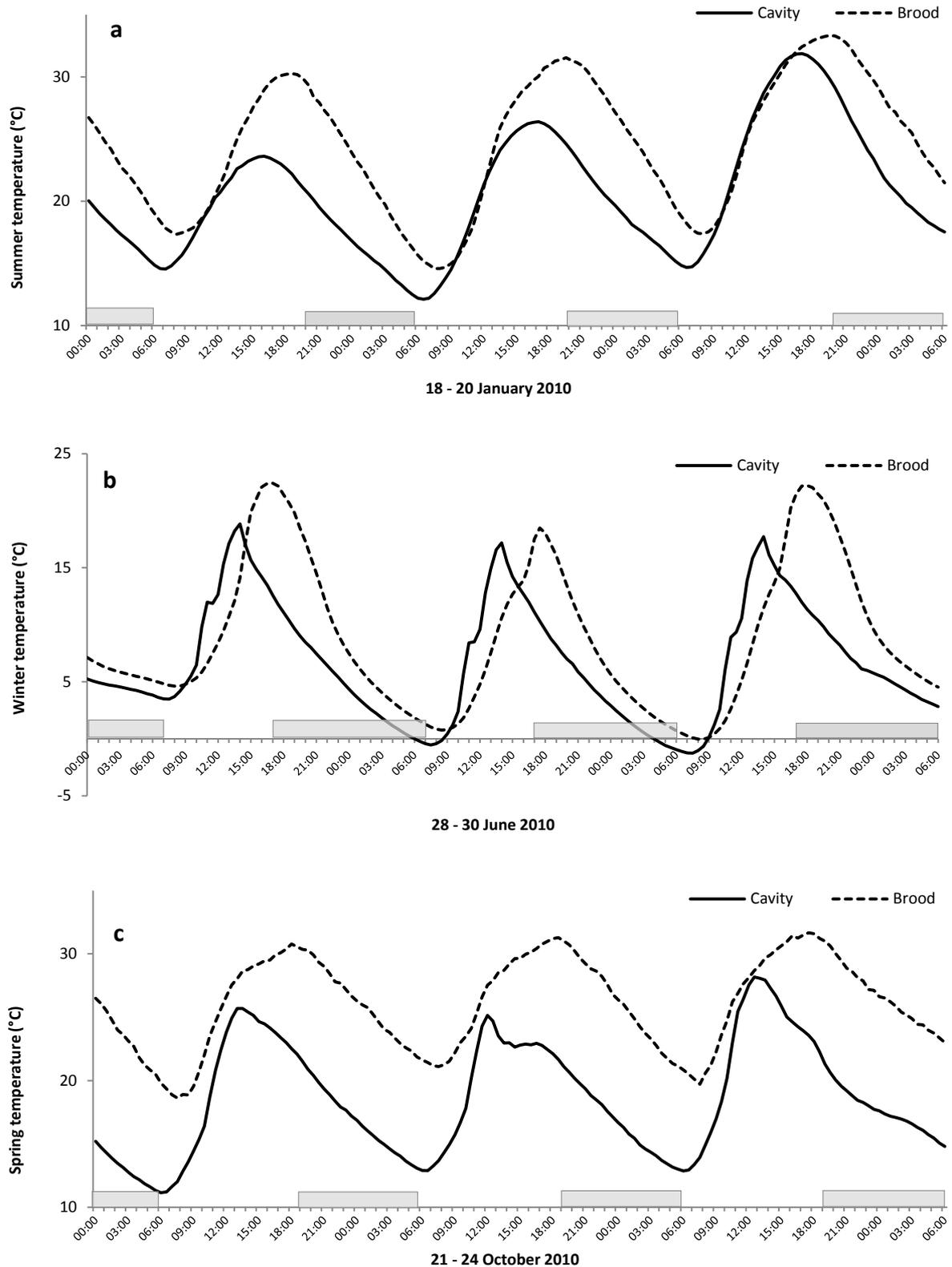


Figure 5 *Austroplebeia australis* brood cluster ($n = 6$) and hive cavity ($n = 3$) temperatures, showing diurnal fluctuations during three consecutive (a) winter, (b) summer and (c) spring days. Shaded areas indicate night hours (sunset to sunrise)

Throughout the year, the greatest differences between cavity and brood

temperatures (up to 12.4°C) were seen toward the end of the day (Fig. 5). In the

early evening, the brood temperatures were higher than the cavity temperatures and remained so until they reached their minimum the next day (Fig. 5). During the morning in summer, autumn and winter, the cavity temperatures were commonly the same as or higher than the brood

temperatures. In winter, cavity temperatures exceeded brood temperatures until mid-afternoon. In spring, the brood temperatures were consistently (96% of all observations) warmer than the cavity throughout the day (Figs. 5c, 6, Table 1).

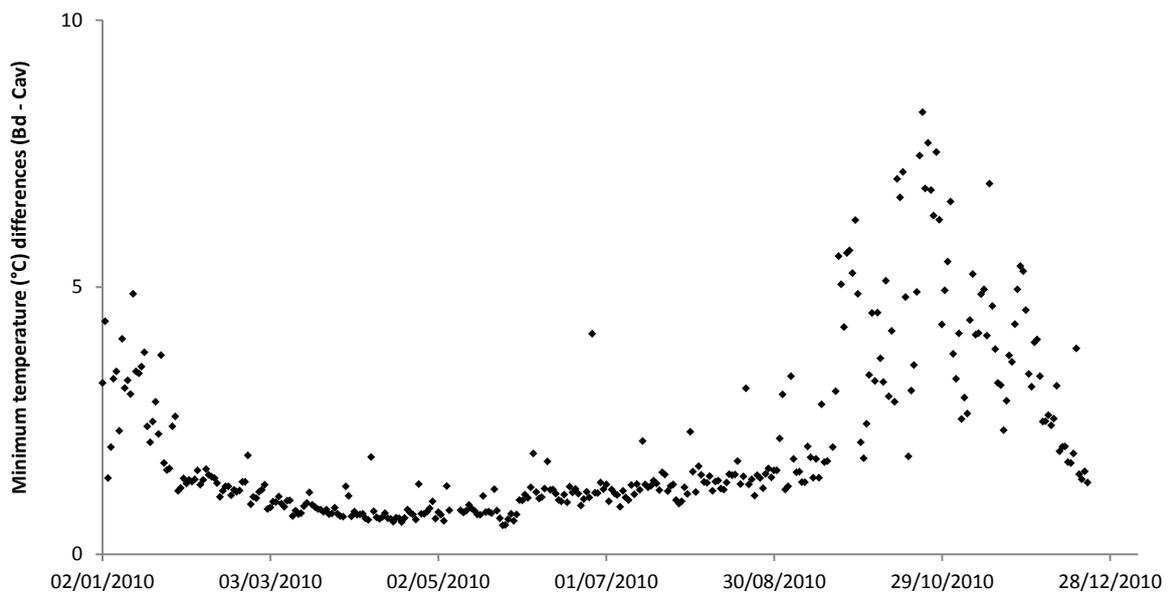


Figure 6 Differences between the daily minimum temperatures in the *Austroplebeia australis* brood cluster ($n = 3$) compared to the hive cavity ($n = 3$) (viz. brood minus cavity) over 12 month period from January 2010 – December 2010

Table 1 Seasonal means and standard errors of temperatures within *Austroplebeia australis* brood ($n = 3$) and hive cavities ($n = 3$) during the 12 months in 2010

Season	Cavity temperature (°C)	Brood temperature (°C)	Difference (°C)	Significance (p)
Summer (28 Dec – 19 Mar)	22.2 ± 0.55	25.0 ± 0.55	2.8	0.023
Autumn (20 Mar – 20 Jun)	16.0 ± 0.34	17.4 ± 0.34	1.5	0.036
Winter (21 Jun – 21 Sep)	12.4 ± 0.73	16.0 ± 0.73	3.6	0.025
Spring (22 Sep – 20 Dec)	18.8 ± 0.85	25.0 ± 0.85	6.3	0.006

While average daily brood temperature was significantly higher than that of the cavity in each of the seasons (Table 1), the largest differences were seen in spring.

The brood was more than 6°C warmer than the cavity in spring and there was a considerable increase in brood size (Fig. 7). In mid-spring (October / November),

there was a substantial increase in the minimum brood temperature compared to the minimum cavity temperature (Fig. 6) In general, when the cavity temperature was $\geq 12^{\circ}\text{C}$ the brood temperature increased by an average of $4.2^{\circ}\text{C} \pm 0.2$, ranging from 0.5 to 12.4°C . When temperatures remained $< 12^{\circ}\text{C}$ the brood temperature increased by an average of

$1.6^{\circ}\text{C} \pm 0.1$, ranging from 0.5 to 7.5°C . When cavity temperatures remained $\leq 7^{\circ}\text{C}$ the brood temperature increased by an average of $1.3^{\circ}\text{C} \pm 0.04$, ranging from 0.8 to 2.1°C . Seasonally, the colonies raised the brood temperature by up to 9.2°C during summer and autumn, by up to 11.8°C in winter and up to 12.4°C in spring.

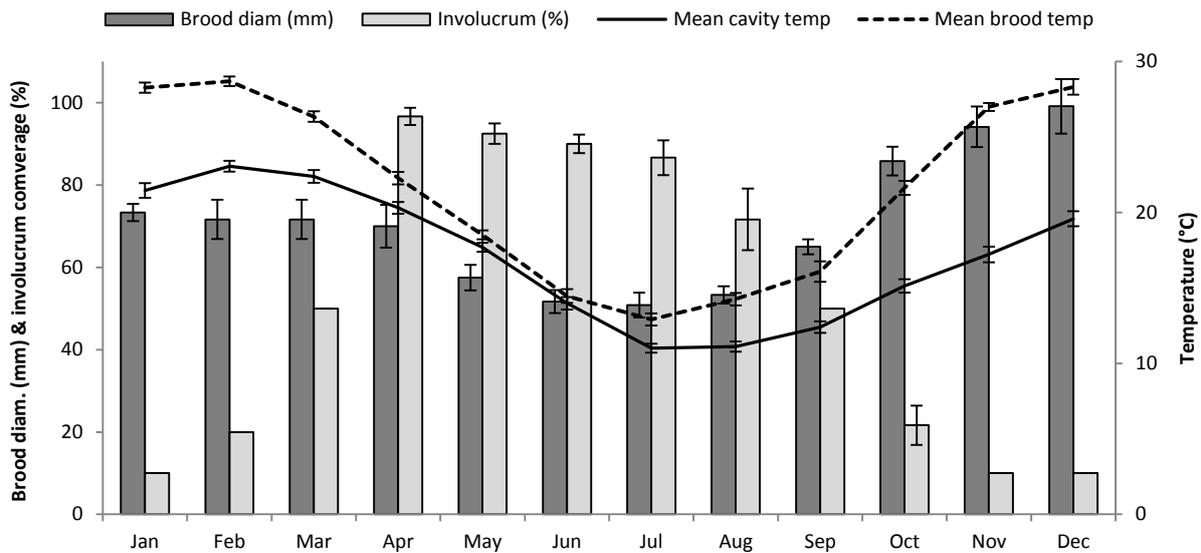


Figure 7 *Austroplebeia australis* brood cluster ($n = 6$) diameter (mm), percentage involucrum cover ($n = 6$), mean monthly cavity ($n = 3$) and mean monthly brood ($n = 6$) temperatures during 2010. Brood diameter decreased as mean monthly cavity temperatures decreased, and increased as temperatures rose. Error bars represent the standard errors of the means

In autumn, as cavity temperatures began to decrease, the involucral coverage over the brood cluster began to expand and by the end of April coverage was almost 100% (Figs 2, 7). The greatest changes in involucral coverage (construction and destruction of lamina) occurred in the two equinox months. The size of the brood cluster also began to decrease in April, remaining unchanged until mean monthly cavity temperatures were $> 12^{\circ}\text{C}$ (Fig. 7). Brood diameter increased and involucral coverage decreased as mean monthly cavity temperatures reached 15°C . Brood size grew by 30% in October and continued to increase with increasing temperatures (Fig. 7).

On warm sunny days flight activities commenced as soon as temperatures were conducive [ambient

$> 20^{\circ}\text{C}$ or cavity $> 18^{\circ}\text{C}$ (Halcroft, 2012)]. In-hive activities such as nectar receipt, nectar dehydration, pollen packing, rubbish removal and guarding increased as temperatures and foraging activity increased. Most foragers returned to the hive before sunset. On warm, but inclement days, foragers did not leave the nest. In-hive tasks such as cleaning, storage pot construction and patrolling were carried out in an unhurried manner but tasks associated with foraging activities were not performed. Some of the brood clusters were constructed directly on the acrylic hive lid. Bees were observed occupying the bee spaces (Fig. 1) between the cells of the brood cluster at night and during the day when it was cold. The majority of bees moved from the nest cavity to the brood chamber during these times.

Throughout the year, the brood clusters contained all stages of developing bees, from egg to pre-emergent pupae. During the coldest months, the presence of larval and pupating brood was confirmed; however, the presence of eggs could not be confirmed non-invasively. Callows were observed throughout the year and the colonies did not become broodless during the winter months.

Laboratory observations on worker activity

Adult worker activity decreased as temperatures were reduced. At 20°C all workers ($n = 10$) within the Petri dishes ($n = 6$) walked quickly. Walking slowed as the temperature was cooled to 17°C and minimal movement occurred at 14°C with only slow antennal movement observed. Bees ceased voluntary movement at 11°C; however, when inverted, they were able to right themselves (quiescent). At 7°C the bees were unable to move (chill coma).

Discussion

Throughout most of the year, the *A. australis* colonies in this study exhibited some degree of thermoconformity, with brood temperatures correlating to the surrounding environmental temperatures. This was most evident by the tracking of brood chamber and hive cavity temperatures in the colder months (Fig. 4c). Brood temperatures closely correlated with cavity temperatures and there was no evidence of brood cooling during the warmest months (Fig. 4a) with the brood remaining warmer than the cavity (Fig. 3).

Throughout the year, the colonies exhibited diurnal temperature fluctuations. However, the temperature peaks within the brood appeared to be driven by colony behaviour. As the day drew to a close and foragers returned to the hive, brood temperatures peaked, independent of the falling cavity temperatures (Fig. 5). This may be due to an increased number of metabolically active foragers entering the colony and remaining within the nest structure. As the evening progressed and the cavity temperature fell, more house bees migrated into the warmer, more comfortable brood structure. Similar phenomena were reported in *Trigona (Frieseomelitta) nigra pauper* (Torres et al., 2009), a South American stingless bee which also builds clustered brood. Although *A. australis* could warm the brood, small increases in brood temperature

were observed when the cavity temperatures were below 7°C; i.e., in spring *A. australis* increased the brood temperature by up to 12.4°C. The reduction in warming capacity in winter could be attributed to the reduced physical activity of the workers when they were exposed to low temperatures, as demonstrated in the laboratory investigation. As cavity temperatures reached 12°C, workers began to move and metabolise at increasing rates, thus increasing their heat output (Figs. 5b, 4c).

The diurnal temperature fluctuations observed (Fig. 5) could in part be ascribed to thermal inertia, given the magnitude of the difference in the temperature between the brood and the cavity and the differences between the seasons (Table 1). However, passive warming due to generation of metabolic heat is the most likely explanation for the difference, as follows.

A 100 mm diameter brood cluster contains approximately 2,500 brood cells (Halcroft, 2012). Given the extended longevity of *A. australis* workers (Halcroft et al., 2013), the adult population is approximately double that of the brood population. Therefore, in a colony with 2,500 brood cells there will be 5,000 adults, 1,000 of which would be foragers (Halcroft, 2012). The cerumen within a 100 mm brood structure, including a layer of involucrum, weighs ~100 g and the provisions within the cells weigh ~100 g (Michener, 1961), and it is assumed that honey bees' wax and *A. australis* cerumen have a similar specific heat capacity (SHC) ($3.40 \text{ j } ^\circ\text{C g}^{-1}$) (Engineering Tool Box, 2013) and the cell provisions (liquid mix of high moisture content honey, pollen and glandular secretions) have a similar SHC to *A. mellifera* honey ($1.7 \text{ j } ^\circ\text{C g}^{-1}$) (Sopade et al., 2006). Extrapolating from a similar sized (6 mg, 4 mm) bee, *Tetragonisca angustula*, the heat production rate of an *A. australis* worker should be around 0.068 mW (Torres et al., 2007). Therefore, to raise the brood chamber temperature by 1°C, the colony needs to produce an energy input of $510 \text{ j} = (3.40 \text{ (SHC wax)} \times 100 \text{ g}) + (1.7 \text{ (SHC provisions)} \times 100 \text{ g})$. In honey bees, the heat output of a colony at night decreases by up to two-thirds that of the daytime output (Southwick, 1982; Fahrenholz et al., 1989) and the Q_{10} of an insect is ~2 – 3 (Keister and Buck, 1974).

In summer during the day (12 h), 1,000 foragers left the hive when the threshold temperature for flight activity was reached ($>18^\circ\text{C}$). If the

remaining 4,000 house bees were to stay within the brood cluster the heat produced would be sufficient to raise the brood temperature by $\sim 23^{\circ}\text{C}$; (4000 bees \times 0.068 mW / 1000 \times 43200 s / 510 j). However, the *A. australis* brood cluster demonstrated a rise of up to 9°C (Fig. 5a), averaging 2.8°C (Table 1). This lower heat output could be explained by the fact that many of the house bees moved away from the brood cluster during the day to perform in-hive tasks such as cleaning, nectar receipt, pollen packing, nectar dehydration, rubbish removal and guarding (Giannini, 1997; Halcroft, 2012). In addition, during summer, there is little or no involucre lamella over the brood (Fig. 7), which would reduce the overall specific heat of the brood and potentially allow heat to dissipate more readily.

In winter, during the day (10 h), since there was a drop in cavity temperature of $>10^{\circ}\text{C}$ we will assume a Q_{15} of 3. This means the winter daytime metabolic rate of a bee was 0.068 mW / 3 = 0.023 mW. All of the *A. australis* bees stayed in the hive because the cavity, and therefore ambient, temperature did not reach the threshold temperature for flight activity. Therefore, the heat produced by the brood cluster during a winter day is calculated as: 5000 bees \times 0.023 mW / 1000 \times 36000 s = 4080 W. This equates to a rise in brood temperature of $\sim 8^{\circ}\text{C}$ above the cavity, which is close to what is seen in Fig. 5b. At night (14 h), metabolic rate is two-thirds less than that of the day; 5000 bees \times 0.008 mW / 1000 \times 50400 s / 510 j, which equates to a rise in brood temperature of $\sim 3.7^{\circ}\text{C}$, mirrored in Fig. 5b and Table 1.

Spring saw the greatest differences between the *A. australis* brood and cavity temperatures (Figs. 5c, 6, Table 1). This may be attributed to an increased population of pre-emergent brood and newly emerged callows, due to an extended developmental period over winter. The ontogenic period is for *A. australis* workers, at 27°C , is ~ 55 days (Halcroft et al., 2013). During autumn and winter, cavity temperatures remained $<12^{\circ}\text{C}$ for 20% of the time. At this temperature, workers would be forced into a low metabolic state of quiescence, which would deepen into a state of chill coma as temperatures dropped below 7°C . As the cavity reached 27°C for very few days (6%) and it is plausible to hypothesise that brood development would have been substantially slowed. As a result of this extended ontogenic time,

the large mass of brood observed during late spring (October – November) (Fig. 7), would have contained a large number of pre-emergent brood and young adults. If we assume that half of the brood mass reached this state and the resulting heat production is calculated as: (4000 + 1250 bees) \times 0.068 mW / 1000 \times 43200 s / 510 j, which equates to a rise in brood temperature of 30.4°C . This figure is much larger than the excess heat (up to 12.4°C) we observed in spring. Again, this may be explained by the rapidly decreasing involucre lamella (Fig. 7) as well as an increase in forager recruitment during spring's floral resource increase.

Involucre construction or deconstruction was the only visible sign that *A. australis* colonies were preparing for seasonal changes. At $>12^{\circ}\text{C}$ the insulative capacity of the cerumen, plus its honey pots, was insufficient to prevent heat loss, with the bees' metabolism rapidly declining as they approached chill coma during the coldest periods (Figs. 4c, 5b). The involucre did, however, encapsulate the brood cluster, thus closing off the bee-spaces between the cells (Figs. 1, 2) and reducing the overall surface area of the chamber. Similarly, wintering honey bees form a tight cluster when temperatures are low ($6 - 8^{\circ}\text{C}$), reducing the surface area, and a loose cluster in summer (Furgala and McCutcheon, 1992). By reducing the surface area of the brood chamber, the involucre constructed by *A. australis* colonies may extend the period when passive warming is available to the developing brood.

Some stingless bee species have been reported to thermoregulate their brood chambers above ambient temperatures (Sakagami, 1982; Roubik and Peralta, 1983; Engels et al., 1995; Moo-Valle et al., 2000; Sung et al., 2008). At ambient temperatures $\geq 15^{\circ}\text{C}$, *T. spinipes* maintained its brood at 35°C (Sakagami, 1982) and *Melipona rufiventris* and *Melipona seminigra* maintained theirs between 30 and 32°C (Roubik and Peralta, 1983). While *A. australis* colonies were unable to maintain temperatures within a narrow range, they did passively heat the brood chamber. The *A. australis* colonies in this study contained quite small brood volumes (70 – 500 mL; equivalent to $\sim 350 - 2,500$ individuals (Halcroft, 2012)) and, in their natural environment, *A. australis* colonies can be found in cavities of between 52 and 109 mm in diameter, with brood volumes ranging from 425 to

2600 mL (Halcroft, 2012). *Trigona spinipes* has an estimated colony population of between 5,000 and 18,000 and in the larger bodied bees, such as *M. seminigra* and *M. rufiventris*, populations range from 4,200 to 7,500 (5 – 10 L) (Sakagami, 1982; Roubik and Peralta, 1983). For these three species, this equates to a substantial heat producing mass, contributing to the internal microclimate of the colony. Sung et al. (2008) showed that *Trigona ventralis hoozana* Strand, thermoregulated its brood between 24 and 31°C when ambient temperatures were as low as 8°C; however, the nest cavity temperature appeared to fall no lower than 12°C. This species has an estimated colony population of 10,000 adults with 12,000 brood cells (Sakagami and Yamane, 1984) and inhabits the cavities of large, living trees which are likely to provide superior insulation. Coupled with heat generated from a massive population, it is not surprising that they are able to maintain elevated brood temperatures during the cooler seasons.

With the exception of Sung et al. (2008), all reports pertaining to this topic have been conducted on colonies that experienced minimum ambient temperatures $\geq 15^{\circ}\text{C}$. Under these conditions, *Frieseomelitta varia* and *Leurotrigona muelleri*, both less populous, cluster-building species which produce no involucre (Engels et al., 1995), are able to increase the brood temperature by only 2 to 8°C above ambient (Sakagami, 1982). At temperatures $\geq 12^{\circ}\text{C}$, *A. australis* was capable of raising brood temperatures by up to 12.4°C above cavity temperature. *Austroplebeia australis* could therefore regulate brood temperature when the cavity temperature was $\geq 12^{\circ}\text{C}$; however, it showed little ability to thermoregulate the brood for cavity temperatures $< 12^{\circ}\text{C}$, probably due to cold-induced quiescence, and exhibited no cooling capacity for high ambient temperatures.

Melipona colimana, a much larger (67 mg, 9.5 mm) South American stingless bee found in the tropical highlands, demonstrated an ability to increase its thoracic temperature when exposed to temperatures as low as 7°C (the lowest reported temperature for the region), and this was accompanied by increased sugar syrup consumption (Macías-Macías et al., 2011). However, during the same study, the adult mortality was 33.6% at 7°C and 50% at 1.7°C. A mortality rate of 100% was observed when pupae of the same species were exposed to 2°C (Macías-

Macías et al., 2011). Our study showed that the adults and brood of *A. australis* were capable of surviving temperatures as low as zero; something that has, thus far, been unreported in any other stingless bee. Further investigation into thermal tolerance in Australian stingless bees is warranted.

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