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Original article

Critical temperatures for survival of brood and adult workers of the giant honeybee, *Apis dorsata* (Hymenoptera: Apidae)

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Abstract – Capped brood (capped within 36 h) and adult workers of *Apis dorsata* were removed from naturally occurring colonies and kept incubated in laboratory hoarding cages at constant temperatures ranging from 26 to 45 °C to study mortality, survivorship, and water and syrup consumption. Capped brood died at temperatures above 36 °C. Below 30 °C adults tended to emerge deformed. Low temperatures delayed development. The optimal temperature for complete emergence of healthy adult workers was 34 °C. Adult workers survived well from about 26 to 36 °C. At 38 °C they died within 5 days and at 45 °C they died within 48 hours. Although syrup (1 sugar: 1 water W/W) consumption did not change over the range of temperatures used, water consumption rose rapidly above 38 °C to over 3 ml/bee in 48 hours at 45 °C. Nest temperature control is critical for survival of brood of *A. dorsata* and adult worker bees have tight constraints on their abilities to endure high temperatures. Water availability is vital for cooling the colony under hot, tropical conditions, and rearing healthy brood.

***Apis dorsata*/ optimal temperature/ thermoregulation/ tropical Asia/ brood/ workers**

1. INTRODUCTION

It is well known that the brood nest of European races of the Western honeybee, *Apis mellifera* L., remains remarkably constant at about 34–35 °C as a result of the thermoregulatory activities of the colony (see references in Seeley, 1985; Moritz and Southwick, 1992; Heinrich, 1993). If capped brood is removed from the brood nest, it can be kept in incubators and the effects of different temperatures on emergence

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of adults and survival can be studied. In general, if the brood is incubated at temperatures even as low as 32 °C, healthy adult worker bees emerge even though development is delayed (Jay, 1959), at 28 °C and below brood dies (Himmer, 1927a, b). If the incubation temperature is elevated by even 1 to 2 °C above 35 °C, the emerging bees are deformed and mortality occurs; above 37 °C, brood death is complete (Himmer, 1927a, b). As is true of most organisms, honeybee pupae have a smaller range of heat tolerance above their optimum than below.

Adult worker bees of European races of *A. mellifera* are capable of withstanding very low temperatures if they can cluster (Allen, 1959), but as individuals they become torpid and immobilized at temperatures of 11 °C and below (Goller and Esch, 1990, 1991). They freeze to death at about 0 °C. The upper levels of temperature tolerance are less well understood. Colonies can withstand temperatures of 45–50 °C for a few hours, but presumably the individual bees are striving to keep their internal temperatures below that level (Allen, 1959; Free and Spencer-Booth, 1962). Above 45 °C enzyme activity and metabolism are likely adversely affected (Seeley, 1985). The Japanese race of the Asiatic hive bee, *A. cerana japonica*, has the remarkable capacity to cause the temperature in a ball of workers to rise to 46 °C in the defense "cooking" of hornet invaders in its nest (Sasaki et al., 1995; Ono et al., 1995). The upper lethal temperature limit for this bee is 48–50 °C (Sasaki et al., 1995; Ono et al., 1995). In clusters of European honeybees, temperatures of about 46 °C have been recorded (Heinrich, 1993) but few studies have been made on the prolonged effects of high temperatures on worker honey bees.

Although open nesting honeybees, such as *A. florea* Fabr. and *A. dorsata* Fabr., are known to thermoregulate, it has been suggested that the effects of temperature on brood maturation may be less pronounced than in cavity nesting species, such as *A. mellifera* and *A. cerana* (Morse and Laigo, 1969). Nevertheless, Mardan and Kevan (1989) have suggested that excessive heat load on colonies of *A. dorsata* results in mass defecation flights (so-called "yellow-rain" (see Seeley et al., 1985)) as the bees dump excess heat and increase their efficiency of thermoregulation to maintain temperatures in the colony within tight tolerances. The brood nest temperatures for *A. dorsata* have been measured at to fluctuate between 30 and 32 °C in the Philippines (Morse and Laigo, 1969) and at about 33.5 °C in India (Viswanathan, 1953) and Malaysia (Mardan, 1989). Thus, we hypothesize that the brood of *A. dorsata* is, like that of *A. mellifera*, highly sensitive to high temperatures and somewhat less sensitive to low temperatures. We also hypothesize that prolonged exposure to excessively high temperatures would cause the death of adult workers even given the opportunity to thermoregulate by evaporating nectar (see Allen, 1959; Free and Spencer-Booth, 1962; Seeley, 1985; Heinrich, 1993 for *A. mellifera*).

To test our first hypothesis, cut portions of capped brood of about the same age (within 36 hours of being capped) were placed in incubators at various temperatures and the emergence recorded. To test our second hypothesis, we placed adult worker bees into incubators with a supply of water and sugar syrup and monitored mortality and duration of survival, and consumption water and syrup, at various temperatures.

2. MATERIALS AND METHODS

Our experiments were made in the laboratory at the Universiti Putra Malaysia, Serdang, Selangor D.E. in July and August, 1986. We used small (34 L nominal volume) portable incubators (Koolatron model P34A; Brantford, Ontario, Canada) with temperature controls within ± 1 to 2 °C accuracy when in a more or less uniform temperature regime (such as in a laboratory) and not packed full to impede internal air circulation (personal communication from D. Paton, Koolatron, 27 St. Catharine Avenue, Brantford, Ontario N3T 1X5). Mercury thermometers were included in the incubators to verify the temperatures within, nevertheless, temperatures in the incubators varied by 1 to 2 °C from place to place depending on the proximity of the heating/cooling element. In the central area of the incubators where our study preparations were placed, we were able to maintain temperatures within ± 1 °C. No water was placed in the incubators to control relative humidity.

Two active and apparently healthy colonies of *A. dorsata* established low on cacao trees in a cacao plantation in Sitiawan, Perak about 200 km from Universiti Putra Malaysia were selected for study because of accessibility and ease of observation. Sealed brood was taken from those colonies by cutting 4 cm × 4 cm sections from the brood area within 36 hours of the brood area having been capped. It was returned to the laboratory and subjected to constant incubator temperatures (± 1 °C) ranging from 30 to 45 °C (30, 34, 36, 38, 40, 45 °C) and kept at ambient temperature (25–28 °C). The number of cells at each temperature ranged from 72 to 100 (Fig. 1). Each piece of sealed brood was maintained in its natural, vertical orientation in a nylon screen cage to capture the emerging adults. The experiments were allowed to run until the brood had completely emerged, or for two weeks after which the remaining brood would have perished.

Groups of 20 adult workers were taken from each of two colonies by aspiration (Mardan, 1989), anaesthetized with carbon dioxide for transport, and kept in hoarding cages (Kulincevic and Rothenbuhler, 1973) supplied with water and sugar syrup (50% W:W) in 20 ml vials, with a piece of empty comb. The cages were placed in the same kind of incubators as noted above and the

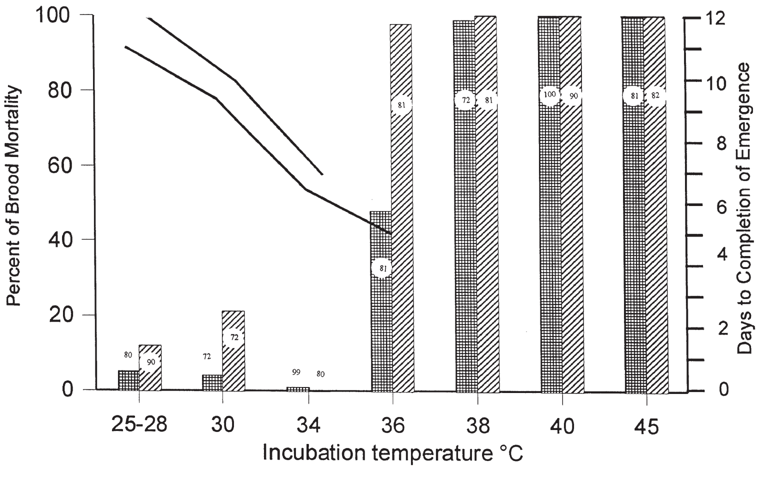

Figure 1. *Histogram bars*: Mortality of capped brood of *Apis dorsata* held in incubators at various temperatures and in ambient laboratory conditions during two experiments (experiment 1 is left-hand bar). The numbers of capped brood cells in each treatment are given in association with each histogram bar. *Lines*: Days to completion of emergence to adults for the two experiments (lower line is for experiment 1) (of emerging adults at 25–28 °C and 30 °C, about 75% had malformed wings).

experiment made in the same manner as described above. Separate experiments were made twice for survivorship and for water and sugar consumption, measured directly by decrease in the amount of liquid in the vials provided.

3. RESULTS

Figure 1 presents the brood mortality in the incubators at different temperatures. At 36 °C brood mortality was over 98% and at 38 °C and above, 100%. At 25–28 °C and up to 30 °C mortality was less than 20 % but almost all the brood that emerged (over 75%) had malformed wings. Only at 34 °C did we obtain minimal mortality (1 or 0%) and apparently well-formed, normal worker bees (Fig. 1).

Figure 2 presents the mortality for adult worker bees. At 40 °C and 45 °C mortality was about 50% at 48 hours and 100% after 72 hours. At 38 °C, all the bees had perished by 120 hours. At temperatures of 36 °C and lower, the bees survived well for up to 7 days. Figure 2 shows that the consumption of syrup rose only slowly with increasing temperature, but that water consumption rose rapidly at temperatures above 40 °C.

4. DISCUSSION

The temperature regimes under which pupae and adult workers of *Apis dorsata* thrive are as constrained as for *A. mellifera*. Capped brood died at temperatures above 36 °C and at temperatures below 30 °C tended to emerge as deformed adults. Lower temperatures delayed development. Himmer (1927a, b) and Jay (1959) found a similarly narrow range for the healthy and optimal development of pupae in *A. mellifera* in Europe and Canada respectively. The optimal temperature for complete emergence of healthy adult workers of *A. dorsata*

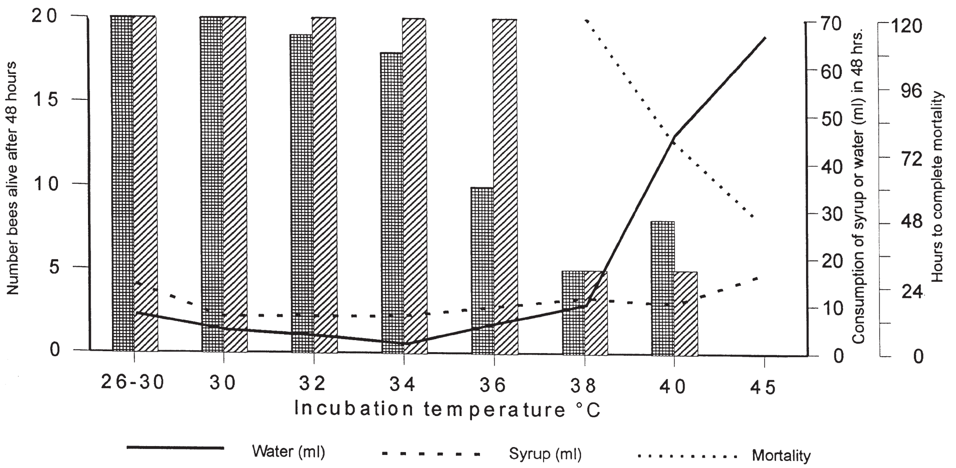

Figure 2. Mortality and duration of survival of twenty adult worker bees of *Apis dorsata,* and their water and sugar syrup consumption rates, held in cages in incubators at various temperatures and in ambient laboratory conditions for 7 days in two experiments. *Histograms*: Survival of 20 bees after 7 days. *Dotted Line*: Hours to complete mortality (at temperatures # 36 °C, most bees survived for the full 7 days of experimentation). *Dashed Line*: Amount of syrup (ml) consumed in 48 hours. *Solid Line*: Amount of water (ml) consumed in 48 hours.

was around 34 °C. This corresponds well with observed brood temperatures in natural colonies (Viswanathan, 1953; Mardan, 1989), but is higher and more constrained that indicated from studies in the Philippines (Morse and Laigo, 1969). For *A. mellifera*, the temperature for optimal development is generally given as 34–35 °C (Seeley, 1985; Moritz and Southwick, 1992; Heinrich, 1993). We were not able to refine our experiments to the needed degree of accuracy with our incubators to test at 35 °C, but at 36 °C we did note some mortality in one experimental colony (Fig. 2). Thus, we conclude that the optimal temperature for pupal development in *A. dorsata* and *A. mellifera* are almost the same. To determine the exact optimal temperature for development for various species of *Apis* would require more sophisticated incubation and temperature measurement in comparative experiments.

Adult workers survived well at temperatures ranging from about 26 to 36 °C. At 38 °C the workers died within 5 days and at 45 °C they died within 48 hours. Again, our results on temperature tolerance with *A. dorsata* are almost the same as those obtained from *A. mellifera* in North America (Allen, 1959) and Europe (Free and Spencer-Booth, 1962). We concur with those authors, and with Heinrich (1981) that water is vital for the bees when they are exposed to temperatures above the optimal brood temperature as is evident from the sharp increase in its consumption in our experiment. Although syrup (1 sugar: 1 water W/W) consumption did not change over the range of temperatures used, water consumption rose rapidly at temperatures above 38 °C to over 3 ml/bee on average in 48 hours at 45 °C (Fig. 2).

It is unlikely that *A. dorsata* living in lowland Malaysia ever experiences temperatures low enough to induce chill-coma, but in other parts of its range (e.g. in montane and more northerly sites) they may and migration to escape cold (and dearth of floral resources) may be important to their survival. Migration by tropical populations of *A. dorsata* seems to be in response to rains and dearth of floral resources (Mardan, 1989).

We conclude that thermoregulation of the brood in the single combs is highly controlled in this open-nesting, tropical, honeybee (as in tropical and temperate zone cavity nesting species) and that developing immature bees and adult worker bees have tight constraints on their abilities to endure high temperatures. Water is a vital resource for cooling the colony under hot, tropical conditions, for rearing healthy brood. It does not seem that life in the tropics and on single-comb, open-air nests permits relaxation (by comparison with cavity nesting bees of temperate and tropical areas) of thermoregulatory activity, although after swarming events, the remaining colonies may be left with rather few bees to incubate the capped brood so development could be delayed, or possibly impaired, depending on the air temperatures at the time. More studies on the temperatures at which chill-coma starts in *A. dorsata* may reveal that for adult workers temperature requirements for activity are stricter than for temperate zone races of *A. mellifera*.

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Résumé – Températures critiques pour la survie du couvain et des ouvrières adultes chez l'abeille géante, *Apis dorsata* (Hymenoptera, Apidae). Dans le cadre d'une étude plus large sur la biologie de la température et la thermorégulation de l'abeille géante *Apis dorsata* Fabr., du couvain operculé de moins de 36 h et des ouvrières adultes ont été prélevés dans deux colonies naturelles qui vivaient près du campus de l'université Putra Malaysia, Serdang, Selangor, Malaisie. Le couvain (morceaux d'environ 4 × 4 cm) et les ouvrières (par groupes de 20) ont été transférés au laboratoire et placés dans des cagettes dans de petites étuves portatives à des températures constantes comprises entre 26 et 45 °C pour étudier la mortalité, la survie et la consommation d'eau et de sirop (1 vol. sucre: 1 vol. eau). Le couvain operculé est mort au-dessus de 36 °C. A des températures inférieures à 30 °C, les adultes qui émergeaient avaient tendance à être déformés. Les températures inférieures retardaient le développement. La température optimale pour l'émergence complète d'ouvrières saines était de 34 °C. Les ouvrières adultes ont bien survécu aux températures comprises entre 26 et 36 °C. A 38 °C les ouvrières sont mortes en cinq jours et à 45 °C elles sont mortes en 48 h. Bien que la consommation du sirop n'ait pas sensiblement changé (8–18 ml/cage de 20 abeilles en 48 h) selon les températures appliquées, la consommation d'eau a augmenté rapidement aux températures supérieures à 38 °C pour atteindre en moyenne plus de 3 ml/abeille en 48 h à 45 °C. Nous en concluons que le contrôle de la température du nid est critique pour la survie du couvain d'*A. dorsata* et que les ouvrières adultes sont limitées par des contraintes strictes en ce qui concerne leur capacité à supporter des températures élevées. L'eau est une ressource vitale pour rafraîchir la colonie dans des conditions tropicales chaudes et pour élever du couvain sain.

***Apis dorsata* / température optimale / thermorégulation / Asie tropicale / survie des ouvrières**

Zusammenfassung – Kritische Temperaturen für das Überleben von Brut und ausgewachsenen Arbeiterinnen der Riesenhonigbiene, *Apis dorsata* (Hymenoptera: Apidae). Als Teil einer größeren Untersuchung zur Thermo-Biologie und zur Thermoregulation bei der asiatischen Riesenhonigbiene, *Apis dorsata*, wurden verdeckelte Brut (verdeckelt innerhalb 36 Stunden) und ausgewachsene Arbeiterinnen aus zwei natürlich vorkommenden Völkern in der Nähe des Campus der Universität Putra Malaysian, Serdang, Selangor, Malaysia, entnommen. Die Brut (Stücke von 4 × 4 cm) und die Arbeiterinnen (in Gruppen von je 20 Tieren) wurden im Labor in Käfigen in kleinen, tragbaren Brutschränken bei konstanten Temperaturen zwischen 26 und 45 °C gehalten. Untersucht wurden Mortalität, Überlebensrate sowie Wasser- und Zuckerwasser-Verbrauch. Verdeckelte Brut starb bei Temperaturen über 36 °C. Bei Temperaturen unter 30 °C neigten die schlüpfenden Tiere zu Deformierungen. Niedrigere Temperaturen verzögerten die Entwicklung. Die optimale Temperatur für den kompletten Schlupf gesunder Arbeiterinnen betrug 34 °C. Ausgewachsene Arbeiterinnen überlebten bei Temperaturen zwischen 26 und 36 °C gut. Bei 38 °C starben die Arbeiterinnen innerhalb von 5 Tagen und bei 45 °C innerhalb von 48 Std. Während sich der Zuckerwasser- (1 Teil Zucker: 1 Teil Wasser) Verbrauch über das getestete Temperatur-Spektrum nicht merklich änderte (8–18 ml/Käfig mit 20 Bienen in 48 Std.) stieg der Wasserverbrauch bei Temperaturen über 38 °C stark auf durchschnittlich mehr als 3 ml/Biene in 48 Std. bei 45 °C an. Wir schließen daraus, dass die Kontrolle der Nest-Temperatur für das Überleben der Brut von *A. dorsata* entscheidend ist, und dass ausgewachsenen Arbeiterinnen enge Grenzen gesetzt sind was ihre Fähigkeit anbelangt, hohe Temperaturen zu ertragen. Wasser ist lebenswichtig, um unter heißen, tropischen Bedingungen das Volk zu kühlen und gesunde Brut heranzuziehen.

***Apis dorsata* / optimale Temperatur / Thermoregulation / tropisches Asien / Arbeiterinnen**

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