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# Individual Acclimatization of *Apis mellifera* L. to the Thermal Homeostasis of the Colony

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**Abstract**

Bees play an important role in maintaining biodiversity by promoting the pollination of numerous plant species. Recent global climate changes are affecting the average air temperature, thereby altering the biological processes of many species. The objective of this study was to evaluate the adaptation of *Apis mellifera* L. bees to temperature increases and their responses to thermal homeostasis in the colony. Research was performed at the Federal University of Paraíba Laboratory of Bees using three treatments: Control, 33 °C and 40 °C. For the latter two treatments, colonies were kept in a 24 m² climate chamber with an opening at the hive entrance, giving the bees access to the outside environment. The following parameters were evaluated: difference between internal and external hivetemperature, thorax surface temperature and total protein concentration in the hemolymph. Internal colony temperature varied according to the external hive temperature. Nurse bees that care for larvae exhibited higher heat production, expressed as thorax surface temperature. Total protein content in the hemolymph was highest in the 40 °C treatment and decreased with ambient temperature. External hive temperature influences internal hive temperature, and nurse bees have higher capacities for thermogenesis.

## Introduction

The greatest economic value provided by bees is pollination, which helps to maintain the planet's biodiversity and helps to increase global agricultural production. Deprivation of this service can negatively affect the sexual reproduction and genetic diversity of plants, as well as compromise the food production and related products (Klein et al., 2007). The monitoring of the pollination dependence of 141 Brazilian agricultural crops – including crops for food, clothing, livestock and biofuel – showed that 85 crops depend on bee pollination. The estimated annual economic input of pollination get close to 12 billion of dollars (Giannini et al., 2015). Another study revealed that 68% of the 53 main Brazilian cash crops are pollinator-dependent. Moreover, the cultivated area (59%) and the monetary value (68%) of pollinator-dependent cash crops are higher than that of non-dependent (Novais et al., 2016), which reinforces the importance of pollinators to the national economy.

However, according to the Intergovernmental Panel on Climate Change (IPCC), the planet is undergoing climate change with significant effects on the environment, biological processes, human health, water resources, agriculture and biodiversity. The main change observed thus far is a significant increase in air temperature that will affect the permanence of many species in their habitats, leading to environmental imbalance. The IPCC report estimates that 20-30% of species will be at high risk of extinction in the case of a 2 to 3 °C rise in average global air temperature (IPCC, 2014). In Brazil, climate change may reduce the probability of pollinator occurrence in up to 0.25 and will affect in up to 100% of the municipalities producers, depending on the crop species (Giannini et al., 2017).

Individual bees are heterothermic insects because they are able to change between ectothermic and endothermic

states through endogenous thermogenic mechanisms, differing from entirely ectothermic insects (Wilmer & Stone, 2004; Stabentheiner et al., 2012). The endothermic warm-up results from the contraction of flight muscles, a mechanism called "shivering thermogenesis" (Heinrich, 1979; Heinrich, 1993; Heinrich & Esch, 1994; Heinrich, 1996). Endothermy episodes in bees are often associated with activities outside the nest (Kovac et al., 2010; Kovac & Stabentheiner, 2011) but social species use their fine thermoregulation ability to control the temperature of the nest or swarm, and for brood incubation (Heinrich, 1981; Southwick & Heldmaier, 1987; Heinrich, 1993; Heinrich, 1996; Bujok et al., 2002; Seeley et al., 2003; Stabentheiner et al., 2003; Jones & Oldroyd, 2007). The precise nest temperature control can be seen as one of the greatest innovations in bee biology, made possible due to the evolution of sociality (Seeley, 2006).

The thermal homeostasis of the colony is determined by polyandrous mating that establishes the genetic diversity of individuals as well as their responses to colony temperature maintenance, integrating behavioral and physiological mechanisms (Jones et al., 2004). Through these mechanisms, social bees can maintain the colony internal temperature between 33 °C and 36 °C, which is ideal for juvenile development and normal behavioral expression (Winston, 2003). Therefore, we intend to investigate the mechanisms of internal temperature control of *Apis mellifera* colonies.

## Materials and Methods

*Location and experimental procedures*

Experiments were conducted at the Laboratory of Bees of the Apiculture and Sericulture Sector, Agricultural Sciences Center, Universidade Federal da Paraíba, Areia, Brazil (6º 58" S, 35º 41" W and 574 m altitude). The climate is tropical semi-humid (As), with rainfall in the autumn and winter, according to the climatic classification of Köppen (Alvares et al., 2013). According to the Brazilian National Meteorological Institute (INMET, 2018), the provisional climatological normals for the study location are as follows: mean annual atmospheric temperature of 22.5 ºC (maximum of 38.2 ºC and minimum of 19.6 ºC), mean annual wind speed of 3.0 m $s^{-1}$, mean annual relative humidity of 82.6%, and cumulative annual rainfall of 1,359 mm.

The animal model was the honeybee (*Apis mellifera* L). Initially, bee colonies were kept in hives manufactured from wood (2 cm thick), measuring 20 × 25 × 50 cm (width × height × length) and containing five frames with honeycomb wax. One side of the brood chamber was replaced by glass – to facilitate the fixation and reading of the thermometer by the observer – covered with black cardboard to promote insulation. As we intended to evaluate the influence of the environmental temperature over internal hive temperature, whether in a controlled (i.e., climatic chamber) or natural environment, no additional insulation was applied to the hives. Eight beehives with similar population densities and queens less than one year old were selected. Each beehive was considered as an experimental unit.

The experimental period was comprised of 60 consecutive days between January and March 2016. Four hives were subjected to different ambient temperatures (33 and 40 °C) inside a climatic chamber. There were 30 days of exposure at 33 °C, followed by 30 days of exposure at 40 °C. The other four hives were exposed to the natural variation of ambient temperature outside the climatic chamber (control group).

The climatic chamber measured 24 m² area, built in masonry, with ceramic floor and PVC ceiling lining. The climatic chamber was heated by infrared lamps (250 W). The bottom board and the hive entrance were connected to an opening in the chamber wall. Thus, bees of the hives placed inside the climatic chamber had free access to the outside, keeping their external activities such as disposing dump waste dump waste products and foraging.

*Relation between internal and external hive temperature*

An analogical thermometer for maximum and minimum (Incoterm, Porto Alegre, RS, Brazil) was installed on the glass side of each brood chamber to monitor the internal ($T_I$, °C) and external ($T_A$, °C) hive temperatures. The temperature data were recorded from 7h00 to 19h00, with a three-day interval between sampling days.

*Body surface temperature*

The body surface temperature ($T_S$, °C) was measured with a thermal image camera (Model Flir TG165, FLIR® Systems Inc., Oregon, USA,temperature range –25 to 380 °C, accuracy of ± 1.5%, resolution of 0.1 °C) that had been calibrated to an emissivity of 0.95. Thermal images were recorded from bees that were standing and performing nursing work (i.e., feeding larvae) over the central brood frames. Images were taken when the camera center point was above the thorax region.

Data collection was performed on four sampling days, with intervals of 15 days between evaluations. At each sampling day, thermal images were recorded from five bees of each experimental unit (i.e., beehives) of each treatment (control, 33 °C, and 40 °C) at each timeof day (8h00, 12h00 and 16h00). A total of 480 bees were monitored.

*Determination of total protein in the hemolymph*

To evaluate the total protein concentrations in the hemolymph, a 40 $cm^2$ section of closed brood comb was removed from each experimental unit – including control group, 33 °C and 40 °C treatments – after 15 days of exposure to the thermal environment. These brood combs were taken to anincubator chamber (Bio-Oxygen Demand type, Model SP-500, SPLABOR, Presidente Prudente, SP, Brazil) that had been calibrated to a temperature of 33 °C and humidity of 80%.

The brood combs remained inside the incubator chamber for 24 hours until the emergence of the bees. Immediately after emergence, 100 bees from each experimental unit were marked on the thorax with organic blue ink and were returned to their original hives. Three samplings were performed after 12 days, and 10 marked bees were recaptured at each sampling.

For hemolymph extraction, the bees were previously anesthetized on ice for 10 minutes. A micropipette was introduced into the dorsal heart through the intersegmental membrane. The hemolymph was aspiratedand then transferred to 0.6 ml Eppendorf tubes. Three drops of a 0.1% phenylthiourea solution (Aldrich-P7629, Grade I, 98%, Merck KGaA, Darmstadt, Germany) were added. Samples were centrifuged at 1,400 rpm for 4 minutes at 4 °C, and the supernatant was removed and frozen at –20 °C. Total protein quantification was performed by the Bradford method (1976), and standard curve construction was performed using bovine serum albumin (BSA). Quantification of samples was performed by spectrophotometry with a wavelength of 595 nm (Cremonez et al., 1998).

*Data analysis*

The study design used was fully randomized with a subdivided plot, where the main plot was the treatment (temperature ranges) and the subplots were the evaluation times. The data were analyzed using the general linear models procedure (GLM) of the software Statistical Analysis System (Statistical Analysis System [SAS], 1999). Between treatment means were compared by the Tukey test with a level of significance set in 5% for all variables. A regression analysis was performed to verify the relationship between internal hive temperature and external hive temperature (i.e. atmospheric temperature) using the OriginPro 8 software (OriginLab Corporation ©, Northampton, MA, USA).

## Results

*External and internal hive temperatures*

The 12-hour variation of the internal and external hive temperature is shown in the Table 1. The $T_A$ mean was 27.83 ± 0.08 °C (n = 2,080). The $T_I$of the control group varied significantly ($p < 0.05$) as a function of the $T_A$, as shown in Fig 1. On the other hand, the beehives maintained at a constant temperature of 33 °C showed no temporal variation in $T_I$, except for at 9h00 (34.35 ± 0.49 °C, n = 40, $p < 0.05$), when the lowest mean temperature was recorded (Fig 2). There was no significant temporal variation in $T_I$ of the beehives submitted to the controlled thermal environment of 40 °C (37.68 ± 0.02 °C, n = 520, $p > 0.05$).

*Body surface temperature*

Treatments and time of day significantly affected ($p < 0.05$) the $T_S$ (Fig 3). There was no significant difference between 33 °C and 40 °C treatments within each time of day. Bees of the control group showed the lowest $T_S$ at all times of day. For thecontrol group and 33 °C treatment, temperatures

**Table 1**. External and internal hive temperature of *Apis mellifera* colonies exposed to natural environmental (control group) and to controlled temperatures of 33 and 40 °C.

| Time | Internal hive temperature (Treatments) | | | | | | | | | External hive temperature | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control group | | | 33 °C | | | 40 °C | | | | | |
| | Mean±SE | Max | Min | Mean±SE | Max | Min | Mean±SE | Max | Min | Mean±SE | Max | Min |
| 7h00 | 28.9±1.59f | 32.0 | 25.0 | 35.72±0.45a | 37.0 | 35.0 | 37.4±0.57a | 38.0 | 36.5 | 24.0±1.85g | 33.0 | 21.0 |
| 8h00 | 29.2±1.57ef | 32.0 | 26.0 | 35.77±0.53a | 37.0 | 35.0 | 37.51±0.49a | 38.0 | 37.0 | 25.75±2.68ef | 35.0 | 22.0 |
| 9h00 | 29.89±1.70ed | 35.0 | 26.5 | 34.35±4.26b | 37.0 | 35.0 | 37.62±0.47a | 38.0 | 36.0 | 28.2±2.97bc | 36.0 | 23.0 |
| 10h00 | 30.29±1.70bdc | 34.0 | 27.0 | 36.07±0.34a | 38.0 | 35.0 | 37.4±0.56a | 38.0 | 36.0 | 29.21±3.16bac | 36.0 | 23.0 |
| 11h00 | 30.83±1.63ba | 34.5 | 27.5 | 36.15±0.49a | 38.0 | 36.0 | 37.35±0.65a | 38.5 | 36.0 | 30.21±2.83a | 36.0 | 24.0 |
| 12h00 | 30.86±1.61ba | 34.0 | 28.0 | 36.22±0.54a | 38.0 | 36.0 | 37.66±0.50a | 38.5 | 37.0 | 30.1±3.18a | 34.0 | 24.0 |
| 13h00 | 30.87±1.60ba | 34.0 | 27.0 | 36.32±0.49a | 38.0 | 36.0 | 37.85±0.36a | 38.5 | 37.0 | 30.3±3.18a | 34.0 | 23.0 |
| 14h00 | 31.05±1.68a | 34.0 | 27.0 | 36.22±0.50a | 38.0 | 36.0 | 37.77±0.50a | 38.5 | 37.0 | 29.56±3.29ba | 34.0 | 22.0 |
| 15h00 | 30.81±1.66ba | 34.0 | 27.0 | 35.12±2.15a | 38.0 | 36.0 | 37.8±0.40a | 38.0 | 37.0 | 29.7±3.77a | 33.0 | 22.0 |
| 16h00 | 30.62±1.56bac | 33.0 | 27.0 | 36.07±0.47a | 37.0 | 35.0 | 37.9±0.33a | 38.0 | 37.0 | 28.03±2.84dc | 32.0 | 22.0 |
| 17h00 | 30.1±1.33dc | 32.5 | 27.0 | 36.1±0.30a | 37.0 | 35.0 | 37.9±0.30a | 38.0 | 37.0 | 26.65±2.43ed | 29.0 | 22.0 |
| 18h00 | 29.71±1.22ed | 32.0 | 26.0 | 36.07±0.42a | 37.0 | 35.0 | 37.9±0.30a | 38.0 | 37.0 | 25.45±1.73ef | 29.0 | 21.0 |
| 19h00 | 29.39±1.24ef | 32.0 | 26.0 | 36.05±0.22a | 38.0 | 36.0 | 38.0±0.30a | 38.0 | 38.0 | 25.05±2.35gf | 29.0 | 21.0 |

Values with different letters in the same column represent significant differences according to the Tukey test ($p < 0.001$)

were lowest at 8h00, highest at 12h00 and intermediate at 16h00. For the 40 °C treatment,the lowest temperature was at 8h00 (32.21 ± 0.22 °C, n = 40), and no differences were observed between 12h00 (34.07 ± 0.31 °C, n = 40) and 16h00 (33.06 ± 0.21 °C, n = 40).

Among treatments, the bees at 33 °C and 40 °C had similar $T_S$ ($p > 0.05$), but their $T_I$ were higher at 40 °C (37.68 ± 0.08 °C, n = 520) than at 33 °C (35.88 ± 0.06 °C, n = 520). As measurements were taken from bees standing on central brood frames, our results suggest that because they are experiencing a higher temperature, they maintained low individual heat production in an attempt to cool the colony and not overheat the internal temperature and damage brood development.

*Total protein concentration in the hemolymph*

The total protein content in bee hemolymph differed between all treatments ($p < 0.05$). As temperature increased, total protein content also increased, as shown in Fig 4.

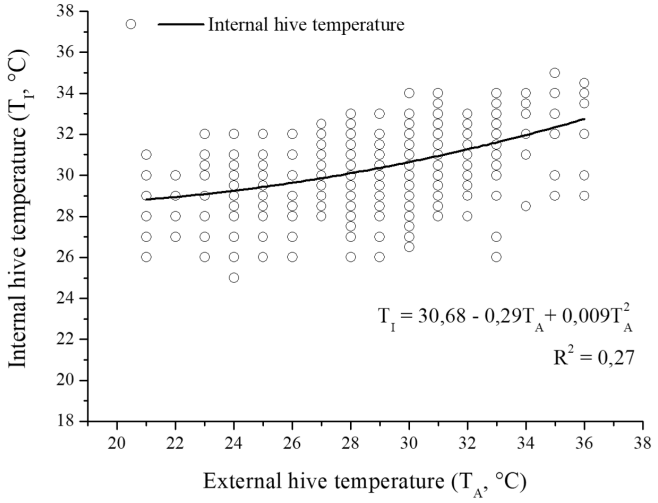


**Fig 1**. Internal hive temperature ($T_I$, °C) of the control group as a function of the external hive temperature ($T_A$, °C).

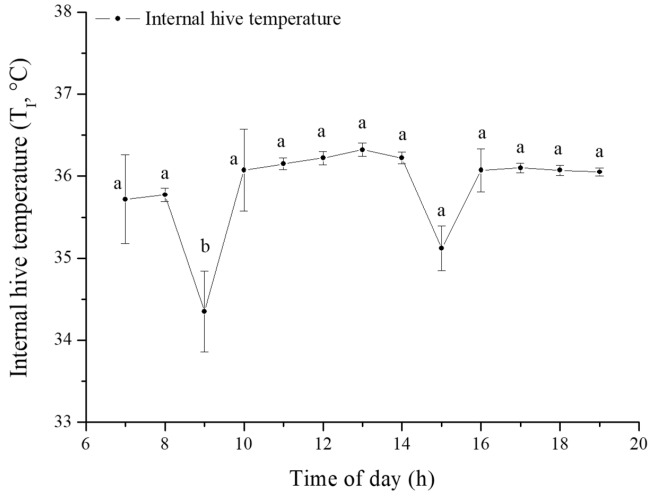


**Fig 2**. Temporal variation of the internal temperature ($T_I$, ° C) of beehives submitted to a 33 °C thermal controlled environment. Different letters indicate a significant difference (Tukey test, $p < 0.05$).

The lowest concentration of total protein in the hemolymph was obtained from the bees of the control group (34.71 ± 1.43 µg $mL^{-1}$, n = 24). The 33 °C treatment revealed an intermediate concentration of total protein (43.35 ± 2.07 µg $mL^{-1}$, n = 12). In the 40 °C treatment bees, a higher ($p < 0.05$) concentration of total protein in the hemolymph was observed (54.48 ± 2.42 µg $mL^{-1}$, n = 12).

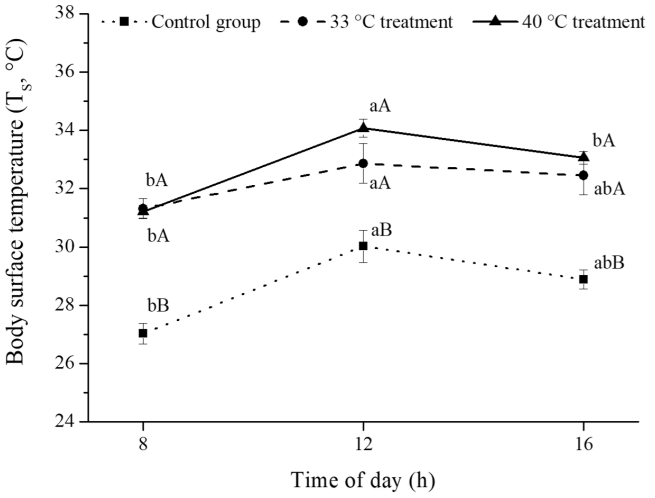


**Fig 3**. Body surface temperature ($T_S$, °C) at different treatment temperatures. Different capital letters in the same time of day and different lowercase letters in the same treatment indicate a significant difference (Tukey test, $p < 0.05$).

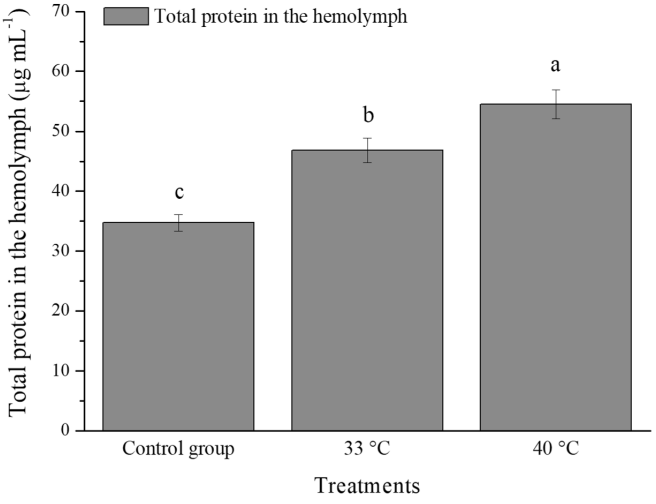


**Fig 4**. Total protein content in the hemolymph of 12-day-old bees at different treatment temperatures. Different letters indicate a significant difference (Tukey test, $p < 0.05$).

## Discussion

The ideal internal temperature of a colony for brood development is between 33 °C and 36 °C (Rosenkranz et al., 1992; Hess, 1926; Himmer, 1927; Dunham, 1929; Tautz et al., 2003). In the present study, colonies of the control group maintained mean $T_I$ below the ideal range. The 33 °C treatment colonies maintained $T_I$ within or slightly above the

ideal range, and the 40 °C colonies maintained $T_I$ above the ideal maximum at all times of day.

A high $T_I$ with a low heat dissipation capacity can seriously damage a bee colony. According to Himmer (1927), who studied the effect of temperature on pupating broods, few bees emerged at temperatures below 28 °C or above 38 °C. Our results show that colonies of the control group reached 28.9 °C at 19h00, and the colonies of the 40 °C treatment reached 38 °C at 19h00, not exceeding the extreme range for pupae malformation. However, the neural malformation of pupae should be considered. The brood rearing temperature may affect the adult brain and the behavioralperformance of the bees, inside and outside the hive (Tautz et al., 2003; Groh et al., 2004). According to Jones et al. (2005), bees pupated below 28 °C or above 36 °C will have a decreased capacity for memory and learning in the realization and recognition of foraging activities. Consequently, the pollination by workers may be impaired.

Our results suggest that the elevation of the thoracic temperature occurred by two different adaptive mechanisms. In the control group, where the average $T_I$ remained below ideal range, the bees possibly activated the muscular heat production. Thermogenesis by muscle vibration is important not only to preflight warm-up (Heinrich, 1979; Heinrich & Esch, 1994; Seeley et al., 2003) but also to improve the bee metabolism (Roubik, 2012), control of the internal hive temperature and brood incubation (Kronenberg & Heller, 1982; Heinrich, 1993; Heinrich, 1996; Kleinhenz et al., 2003). The heat production rates are caste-dependent; workers bees contribute more than drones and queens for hive thermoregulation (Fahrenholz et al., 1989).

When the internal hive temperature exceeded the ideal range for brood development (i.e., 33 and 40 ° C treatments), workers probably sought to intensify the convective heat loss by fanning over the brood area (Southwick & Heldmaier, 1987; Southwick & Moritz, 1987; Starks & Gilley, 1999). According to Bordier et al. (2017), heat-challenged workers bees show a nurse-like profile as a result of increased expression of specific genes. The activation of fanning behavior and the ability of nest cooling by workers arepositively influenced by intracolonial genetic diversity (Jones et al., 2004; Graham et al., 2006; Jones et al., 2007).

Adade and Cruz-Landim (2004) investigated the aging of the flying muscles of newly emerged bees, nurse bees and forager bees in the genus Apis. They found that the muscle fibers of nurse bees have larger diametersthan those of newly emerged bees and forager bees. Nurse bees, compared with newly emerged bees and forager bees, also have larger amounts of stored glycogen. These results indicate that nurse bees have the highest individual capacities for thermogenesis and contribute most to thermal homeostasis. Adade and Cruz-Landim (2004) have shown that nurse bees have higher levels of activity and greater capacity to generate metabolic heat by contracting their thorax muscles and generating adenosine triphosphate.

Our data on concentrations of total protein in the hemolymph are in agreement with those of Crailsheim (1992), who studied colonies throughout the year and observed that the amount of total protein in the hemolymph changes seasonally. This author observed that protein content is lower during the winter and tends to increase during the spring. Protein content also decreases with age due to less use according to activity. Forager bees perform locomotor activities and do not require protein. However, protein content is higher in young bees up to 12 days of age (nurse bees) and even higher with increasing temperature, as temperatures are higher in spring than in winter.

The main function of hemolymph is the distribution of available nutrients from the digestion process and the receipt of metabolic products (Rocha et al., 2003). The protein present in the hemolymph of nurse bees is metabolized as a storage protein (Amdam and Omholt, 2002). This storage remains in the fat body, which is a central metabolic organ for these insects (Arrese & Soulages, 2010). According to Amdam and Omholt (2002), protein stored by the fat body is used in several vital processes, such as the maintenance of tissues, and represents a source of energy through the catabolism of glycogenic amino acids that are released into the hemolymph when necessary. According to the results of the present study, the lower amount of protein in the hemolymph at lower temperatures can be explained by the immediate use of this nutrient by nurse bees for heat generation, or by storage to prolong the thermogenic capacity. This study measured the concentration of protein dispersed in the hemolymph, disregarding the fat body, which was discarded after sample centrifugation.

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