Intranidal temperature and body size of Africanized honey bees under heatwaves (Hymenoptera: Apidae)

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Abstract – It is generally accepted that temperate subspecies of honey bees can maintain stable temperatures inside their nests; however, little information is available on the cooling ability of tropical honey bees and the effect of high environmental temperatures on individuals. In this study, we registered temperatures in the brood area of strong- and medium-populated colonies of Africanized honey bees during heatwaves (maximum environmental temperature 44 °C) between April and May in the tropical Yucatán Peninsula of México. To evaluate the effect on colonies, we compared the body size of workers produced under high temperatures in the field and siblings produced at stable 34–35 °C laboratory conditions. We found that in the field, temperatures of the brood nest in both types of colony can increase above 36 °C during the hottest part of the day, especially in less strong colonies. Workers produced in the field were significantly smaller, compared to those reared in the lab at stable temperatures. This is a first study reporting body size reduction in honey bees as a consequence of possible thermoregulation disruption during heatwaves. We discuss our results considering potential effects of heat stress to Africanized honey bee colonies and tropical beekeeping.

thermal stress / climate change / tropical beekeeping / Yucatan Península / colony fitness

1. INTRODUCTION

Pollinators are increasingly under threat from human activities, including global warming. Several predictions state that the increase of the world's mean temperature by the end of the twenty-first century could be in the range of 0.3 °C to 4.8 °C (IPCC 2014).

The Western honey bee (*Apis mellifera*) is notable among insects because of the ability of effectively controlling brood temperatures within narrow variation of 34–36 °C (Kronenberg and Heller 1982; Ritter 1982; Southwick 1987; Heinrich and Esch 1994). It is generally believed that compared with ectothermic aphids, honey bees can mitigate the negative impact of global warming due to the capacity of maintaining nest stable temperature by thermogenesis or cooling (Le Conte and Navajas 2008; IPBES 2016).

In general, honey bee nest thermoregulation has been more extensively studied in temperate environments, focusing more on aspects of nest heating rather than cooling it down (Bujok et al. 2002; Kleinhenz et al. 2003). The natural

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distribution of *A. mellifera* encompasses a wide variety of environments, including desertic, tropical, and temperate regions where multiple subspecies or “races” have evolved, showing contrasting differences in behavior and adaptation to their environments (Winston 1987; Ruttner 1988). Because of differential adaptation, subspecies contrast in their ability for temperature regulation and survival in temperate and hot environments (Abou-Shaara et al. 2017). For instance, it is known that tropical subspecies, like *A. m. scutellata* from Africa, exhibit reduced ability to survive at low temperatures and accordingly its natural southern distribution is limited to the 34 °C (Hepburn and Radloff 1998). Neotropical Africanized honey bees (AHB, a hybrid between *A. m. scutellata* and resident European honey bees, EHB) also exhibit latitude and altitude distribution limited by low temperatures (Sheppard et al. 1991; Quezada-Euán et al. 2003). Similarly, temperate subspecies have reduced adaptation and survival in hot climates (Abou-Shaara et al. 2017).

Few records of nest temperatures in honey bees have been made in the neotropics. In a preliminary study in Venezuela using one AHB and one European colony, it was found that nest temperatures in the hottest part of the year could reach above 39 °C in both (Villa et al. 1987). Moreover, how heat stress may affect honey bee colonies in tropical environments has been little investigated.

When nest overheats, workers engage in behaviors such as partial evacuation, in an attempt to reduce excess temperature (De Almeida 2008; Stabentheiner et al. 2010). This may leave immature stages (eggs, larvae, and pupae) unprotected from high temperatures for some periods, but the consequences have not been determined. Recently, it was found that AHB workers submitted to fluctuating high temperatures in the lab (maximum of 40 °C for 1 h) during the pupal stage resulted in an early onset of foraging and a consequent shorter life span compared to siblings raised at 34–35 °C (Medina et al. 2018).

The tropical Yucatan Peninsula of Mexico is among the top beekeeping areas in the world (Echazarreta et al. 1997). Indeed, the most productive beekeeping is practiced in the tropical and subtropical areas of Mexico where AHBs are predominant (Quezada-Euán 2007; Domínguez-

Ayala et al. 2016). Environmental temperatures in the Peninsula can reach up to 45 °C during the dry season between April and June (Poot-Báez 2018). Understanding the effect of prolonged exposure to high temperatures is important for predicting the possible consequences of climate change on tropical honey bees and the livelihood of people who depend on apiculture in these regions (Oldroyd and Wongsiri 2006; Bradbear 2009; Moritz and Erler 2016; Switanek et al. 2017).

In this study, we recorded temperatures in the brood area of AHBs colonies under field conditions during heatwaves (April–May) and compared the body size of workers exposed to high environmental temperatures during the pupal stage with individuals from the same colonies maintained at stable temperatures in the lab. This type of investigation is basic for understanding the dynamics of nest thermoregulation in tropical bees and for planning strategies to mitigate the impact on imminent global warming on beekeeping in tropical regions.

2. MATERIALS AND METHODS

The study was conducted at Xmatkuil, 15.5 km south of Merida, Yucatan, Mexico, over a period of 3 months from March 2017 to May 2017, encompassing the dry and hottest season in the Yucatan. Environmental temperatures during this period can reach 42–43 °C for periods of a few hours per day and for several continuous days (Poot-Báez 2018).

Ten colonies kept in Langstroth hives were selected from commercial apiaries after being confirmed as Africanized. Colony origin was confirmed using the Fast Africanized Bee Identification System (FABIS), measuring the legs and wings of ten worker honey bees per colony (Rinderer et al. 1987). We selected colonies with mean FABIS probability of being Africanized of 0.99.

The population of each colony was estimated using the surface area occupied by adult workers in accordance to the figures by Delaplane et al. (2013) and the classification as strong (80–100% total surface of comb occupied), medium (60–79% of surface comb occupied), or weak (less

than 60% of comb area occupied). We selected five medium populated (MP) colonies and five strong ones (SP). Temperature of the brood area in each colony was registered with the help of dataloggers (Extech® RHT10 and Sato® SK-L200TH II α). In each experimental colony, we placed one datalogger between the two central brood frames. The temperature of the brood area was registered continuously at 5 min intervals for 24 h three times per week during 3 weeks. An additional datalogger was placed in an empty hive in the same apiary to monitor environmental temperatures. Environmental temperatures in the shade were obtained from a local weather station too. The temperature between population strength (PS), colony, and PS-colony was compared using ANOVA for a two-factor factorial design in blocks (Montgomery 2013), followed by Tukey's multiple comparison test using STATGRAPHICS Centurion 18 (Statgraphics 2018) statistical software.

To evaluate the effect of extreme temperatures on the development of worker bees, we selected brood from the five MP colonies. We selected capped pupae (white pupae) after a heatwave between the 23rd and 28th of May. A comb section of ca. 70 cells was taken to the lab, while other section from the same comb (also ca 70 pupae) was also cut off but left in the hive during all the development of individuals. The comb sections in the lab were kept in wooden boxes at controlled conditions (34° to 35.5 °C and 75% humidity) in an environmental chamber (Binder KBF 720).

The comb sections were monitored to detect when worker bees started to emerge. Samples of emerging bees were collected from field and lab combs and kept in ethanol. Between 10 and 20 workers were dissected from each treatment colony. The right forewing, hind leg, head, and thorax were mounted on glass slides to be photographed using a Leica S8 APO microscope. Four morphometric variables associated with body size in bees were measured using ImageJ (Rasband 1997-2012) on each worker: head width, intertegular width, forewing length, and tibia length. A GLM procedure of the statistical software SPSS (2006) was used to compare the size of the different structures between treatments. Additionally, a principal component analysis

(PCA) was used to obtain parameters of overall body size combining the four morphometric traits measured on individual bees. PCA reduces the total variation of the data to a few variables called components. The correlation matrix procedure was selected as the resulting principal component1 (PC1) is better related with body size (Wiley 1981). The resulting coefficients for each PC were used to calculate scores as individual measures of body size. The individual scores of PC1 (related with body size) of field and lab workers were compared using the GLM procedure with treatment and colony and their interaction as the main factors.

3. RESULTS

The highest recording of environmental temperature in the empty hive was 44.3 °C, during a heatwave between the 23rd and 28th of May. The average and maximum temperatures recorded in MP colonies during this period were 36.04 ($\pm$ 1.2) °C and 39.4 ($\pm$ 1.7) °C. For the SP colonies, the average and maximum temperatures were 35.2 ($\pm$ 0.8) °C and 37.5 ($\pm$ 1.0) °C, respectively (Figures 1 and 2). Average and maximum environmental temperatures in the shade were 32.5 ($\pm$ 4.7) °C and 39.7 ($\pm$ 4.4) °C. The comparison of temperatures between MP and SP colonies was statistically significant ($F_{1,1296} (0.01) = 926.95$; Supplementary file 1). The ANOVA also revealed an effect of colony ($F_{4,1296} (0.01) = 66.82$, $P < 0.001$) and the interaction between population strength and colony ($F_{4,1296} (0.01) = 125.09$, $P < 0.001$). Notably, recordings of nest temperatures in the nests of MP colonies during the heatwave reached 40 °C but only for 1 h in average (between 15 and 16 h), a value much higher than those considered safe for brood development (Figure 2). In comparison, temperatures in highly populated ones in the same period of time were also above the 35 °C but did not go over 39 °C (Figure 2). In general, nest temperatures fluctuated more in MP colonies compared with SP ones (Figure 2). During the highest temperatures, we observed nest evacuation of adult population more frequently and in more numbers in SP colonies compared with MP ones.

Figure 1. Average mean temperatures per colony (numbered 1 to 5) for the week between 23rd and 28th of May 2018 during a heatwave in SP (A) and MP (B) groups, together with environmental (ENV) temperatures. The ends of each box are the upper and lower quartiles; the whiskers represent the average of the highest and lowest temperatures. The red line represents the average temperature of the colonies in each group.

Regarding the body size of workers reared under heatwave conditions in the field and stable conditions in the lab, it was found that the former were significantly smaller in all four traits measured (Table 1, Supplementary file 2). We used the PC1 and PC2 coefficients (Supplementary file 3) to calculate individual scores. The mean of scores for PC1 was significantly smaller in field bees (Figure 3; Supplementary file 2). It is generally accepted that PC1 reflects body size (Wiley 1981) and, thus, the values of scores of field bees for that component confirm their overall smaller body size (Table 1; Figure 3). In addition to the effect of treatment, colony also had a significant effect on intertegular distance ($F_{4,165} (0.01) = 6.62$), forewing length ($F_{4,165} (0.01) = 29.4$), and tibia length ($F_{4,165} (0.01) = 13.8$). Likewise, there was a significant effect of colony on PC1 ($F_{4,165} (0.01) = 17.7$). The interactions between colony and treatment were

significant only for forewing ($F_{4,165} (0.01) = 11.32$) and tibia length ($F_{4,165} (0.01) = 5.02$).

4. DISCUSSION

We found that during heatwaves, the nest temperature of colonies of AHBs can significantly increase above those considered normal for development of individuals in temperate regions. Notably, the nest temperature of MP colonies increased significantly more compared with SP ones. It was also found that pupae exposed to high environmental temperatures in the field (12–13 days) resulted in smaller workers compared with those reared in the lab, under conditions considered stable for development.

Our results provide evidence that social buffering of honey bee colonies in tropical conditions may be challenged during heatwaves. However,

Figure 2. Average mean temperatures per hour in both groups SP (A) and MP (B) of colonies for the week between 23rd and 28th of May 2018 during a heatwave, together with environmental temperatures. The whiskers represent the average of the highest and lowest temperatures.

the temperature of the nests increased only in the hottest hours of the day, particularly in MP colonies, returning to those considered normal after. AHB workers kept in shaded hives were able to maintain their body temperatures at relatively normal levels, but this ability is hindered at environmental temperatures above 36 °C causing almost linear increases of body temperature (Domingos et al. 2018). Thus, thermoregulation mechanisms can be disrupted above certain environmental temperature (in our study ca. 43 °C) causing the colonies to keep pace with rising outdoor temperatures. Probably to compensate temperature increase during the hottest part

of the day, colonies evacuated the hive, and this was more frequent in SP colonies, presumably leaving immature stages exposed to high temperatures. However, contrary to expectations, the temperature of the brood in SP colonies was significantly more stable during these hours compared with MP ones. In general, the temperature of MP colonies varied more than the SP ones. Therefore, SP colonies seem to better thermoregulate the brood under heatwaves. Heat-dissipating mechanisms may be performed more efficiently in colonies with numerous workers. In colonies exposed to heat stress, workers collect and deposit water on combs and cells, while

Table 1. Comparison of means for morphometric characters and scores for principal components (PC1 and PC2) of AHB workers reared under controlled temperature conditions (lab) and in the field exposed to heat stress (field). **denotes significant at $P < 0.01$; NS denotes not significant at $P < 0.01$.

			Treatment		
Character	Lab (<i>n</i> = 50)		Field (<i>n</i> = 91)		<i>F</i> value
	Mean	St err	Mean	St err	
Head width (mm)	3.681a	0.012	3.602b	0.006	26.8**
Intertegular distance (mm)	2.777a	0.015	2.730b	0.008	8.03**
Forewing length (mm)	8.600a	0.028	8.239b	0.015	128.3**
Tibia length (mm)	2.697a	0.015	2.569b	0.008	59.04**
PC1	0.769a	0.138	−0.307b	0.077	82.64**
PC2	0.099a	0.159	−0.248b	0.088	6.17 NS

Figure 3. Distribution of principal components 1 and 2 (PC1 and PC2) score values of AHB workers reared at stable temperature in the lab (blue triangles) and under heat stress in the field (red triangles).

others create air currents by fanning their wings to eliminate heat by water evaporation and convection (Jones and Oldroyd 2007). In addition, although part of the population empty the nest to reduce the heat produced by metabolism, some workers remain inside acting as shields against excessive heat (Siegel et al. 2005). Shield workers move more intensely to the hottest area in the hive, to create air currents and dissipate heat and/or to absorb it and move it to less heated areas in the nest (Siegel et al. 2005). Notably, the initiation and thresholds of thermoregulatory behavior seem to depend on the population of colonies (Starks and Gilley 1999; Cook and Breed 2013; Cook et al. 2016). In addition, nest cooling requires that different workers engage in several activities at the same time (Egley and Breed 2012). Therefore, colonies with strong populations could engage faster and more efficiently in nest cooling because of the large number of workers available. Weak colonies, on the contrary, may assign less numbers of individuals to each of the different mechanisms to reduce nest overheating or they may concentrate in only a few of these, resulting in less efficient thermoregulation of the nest.

High environmental temperatures during worker development can affect several traits of the

resulting individuals, from physical and behavioral abnormalities to death in extreme cases. In contrast with adults, honey bee brood is stenothermic, and accurate temperature regulation is needed for proper development. The pupae are even more sensitive to temperature changes compared with earlier stages (Stabentheiner et al. 2010). Nest temperature can affect the rate of development of individuals. In temperate honey bees, slow development occurs when temperatures reduce below normal (Fukuda and Sakagami 1968) and presumably accelerates when nest temperatures rise. In ectotherms, a general increase of physiological processes occurs at higher temperature resulting in fast developing individuals and smaller body size (Chown et al. 2002). An accelerated metabolism and consequent faster development of the larvae in overheated nests may explain why workers under field conditions were smaller than those reared at constant temperatures in the lab. To our knowledge, this is the first time that high temperatures in the field are linked to body reduction in honey bees, as these are generally regarded as efficient thermoregulators.

The reduction in size of the different measured traits in field bees was less than 5% compared with those reared under lab conditions. It would be

adventurous to predict the effect of such magnitude of reduction in body size of individual workers on colony fitness and productivity. However, it is known that several components of worker body size are important for colony fitness. Pupal weight, corbicular size, and proboscis length have been correlated honey production in temperate honey bees (Milne Jr. 1980; Milne Jr. and Pries 1984). In AHBs, honey production is also influenced by pupal weight (Souza et al. 2002; Zárate et al. 2008) and significantly correlated with the corbicular area (Souza et al. 2002). Indeed, smaller bees may have limited capacity to carry large or heavy loads (Milne Jr. and Pries 1986), reducing the opportunity of colonies to accumulate large reserves which in turn may compromise colony reproduction and survival, as well as productivity.

Important traits other than body size may also be affected in AHB workers reared under heat stress. In temperate honey bees, the transition from in-hive to outdoor activities, longevity, and communication can be altered in workers that develop at abnormal high temperatures (Tautz et al. 2003; Jones et al. 2005; Becher et al. 2010; Abou-Shaara et al. 2017). Similarly, AHB pupae reared at fluctuating high temperatures in the lab showed higher rates of body asymmetry and an early start of foraging coupled with shorter life span (Medina et al. 2018).

Although potential negative effects may be associated with nest overheating in individual AHBs workers such as shorter life span (Medina et al. 2018), and in our study body size, more evidence is needed to understand of the ultimate effects on colony fitness and productivity. Indeed, the adaptation of different subspecies to their historic climate conditions is well-documented (Villa et al. 1987; Abou-Shaara et al. 2012; Kovac et al. 2014; Alattal and Alghamdi 2015; Abou-Shaara et al. 2017), and it may be possible that AHB colonies could better stand increases in environmental temperatures with less negative effects compared with EHB. It should also be considered that AHBs in tropical and subtropical conditions may exhibit several adaptations over their temperate counterparts, such as disease resistance (Martin and Medina 2004) and active foraging at high temperatures (Heinrich 1979). Nonetheless, measures should be taken to protect hives from

extreme heat. Colonies kept in shade potentially invest less energy in thermoregulation compared with those kept in direct sun light, which may result in differential productivity (Domingos et al. 2018).

Our results indicate that AHBs colonies exposed to high environmental temperatures can experience an increase in nest temperature and workers could become smaller as a result. Notably, strong colonies can better control those increments. Recommendations to ameliorate possible negative effects should be directed to maintain colonies with strong populations and to provide them with adequate shade and nearby water during the hottest months of the year. In addition to the risks of disease transmission and genetic erosion, introduction of stocks from temperate areas does not seem a plausible strategy for tropical beekeeping under the eminent effects of global warming. We think a better option for tropical and subtropical Mexico would be to select/reproduce honey bees already adapted to local conditions and take advantage of their tolerance to heat.

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Température à l'intérieur du nid et taille corporelle des abeilles africanisées lors des vagues de chaleur (Hymenoptera : Apidae).

Stress thermique / changement climatique / apiculture tropicale / péninsule du Yucatan / bonne santé de la colonie.

Temperatur innerhalb des Nests und Körpergröße von afrikanisierten Honigbienen bei Hitzewellen (Hymenoptera: Apidae).

Thermischer Stress / Klimawandel / Bienenhaltung in den Tropen / Yucatan-Halbinsel / Fitness des Bienenvolks.

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