

Hannelie Human · Sue W. Nicolson ·
Vincent Dietemann

Do honeybees, *Apis mellifera scutellata*, regulate humidity in their nest?

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Abstract Honeybees are highly efficient at regulating the biophysical parameters of their hive according to colony needs. Thermoregulation has been the most extensively studied aspect of nest homeostasis. In contrast, little is known about how humidity is regulated in beehives, if at all. Although high humidity is necessary for brood development, regulation of this parameter by honeybee workers has not yet been demonstrated. In the past, humidity was measured too crudely for a regulation mechanism to be identified. We reassess this issue, using miniaturised data loggers that allow humidity measurements in natural situations and at several places in the nest. We present evidence that workers influence humidity in the hive. However, there are constraints on potential regulation mechanisms because humidity optima may vary in different locations of the nest. Humidity could also depend on variable external factors, such as water availability, which further impair the regulation. Moreover, there are trade-offs with the regulation of temperature and respiratory gas exchanges that can disrupt the establishment of optimal humidity levels. As a result, we argue that workers can only adjust humidity within sub-optimal limits.

Introduction

Honeybee colonies show efficient regulation of the biophysical parameters of their hive. Constant temperature is crucial for the normal growth and development of the immature stages (Himmer 1927; Degrandi-Hoffman et al. 1993). Colony thermoregulation is well-studied in honeybees, and hive temperatures are adjusted through various mechanisms. During winter, honeybees form clusters to

conserve heat generated by the shivering of their flight muscles (e.g. Stabentheiner et al. 2003). During summer, when the nest temperature exceeds the optimum range, workers collect water and spread droplets on the comb; fanning causes their evaporation and results in active cooling (Lindauer 1955). Water is collected either by specialised foragers or incidentally through foraging for nectar (Lindauer 1955; Kühnholz and Seeley 1997).

In spite of the supposedly important role of humidity in brood development (Park 1949; Lindauer 1955), little is known about how this parameter is regulated by honeybees, if at all (Ribbands 1953; Büdel 1960; Simpson 1961; Johansson and Johansson 1979; Willmer 1986). Earlier measurements of humidity were made in hives emptied of half the frames and occupants, or in an extra compartment placed on top of the hive, to accommodate large monitoring devices, such as hygrothermographs (e.g. Oertel 1949). Usually, the measurements were of relative humidity, which is dependent on temperature (as the saturation vapour density of water in air increases with air temperature), and this led to the conclusion that humidity in beehives simply follows variations in temperature and that bees do not actively regulate it (Lindauer 1955; Simpson 1961). We have investigated whether honeybees regulate humidity in their hives using miniaturised technology that made it possible to measure this parameter in a biologically relevant manner.

Materials and methods

We measured temperature, absolute humidity (AH) and relative humidity (RH) inside three *Apis mellifera scutellata* colonies. Each contained approximately 20,000 bees and was housed in a Langstroth hive with one shallow super. AH was measured to exclude the effect of temperature and assess the water vapour density in the hive atmosphere. The apiary was located in the Roodeplaat Nature Reserve, Gauteng Province (28°39' E, 25°66' S), in the summer rainfall area of South Africa. Monitoring occurred in the dry winter month of July 2005, during peak

H. Human (✉) · S. W. Nicolson · V. Dietemann
Department of Zoology and Entomology,
University of Pretoria,
Pretoria 0002, South Africa
e-mail: hhuman@zoology.up.ac.za
Tel.: +27-12-4204872
Fax: +27-12-3625242

nectar flow of *Aloe greatheadii* var *davyana*. These conditions are ideal for our study as the dry atmosphere creates a stress to which colonies have to react, but the presence of abundant forage ensures that the colonies are healthy and can adjust to this natural stress. The hives were within 1 km of a dam, providing them with a source of water.

Miniature HOBO H8 data loggers (61×48×20 mm, Onset Computer Corporation, Pocasset, MA, USA) were used for continuous recording of temperature, AH and RH (at 2-min intervals for 4 consecutive days). The operating ranges of the loggers for RH, AH and temperature are 25–90%, 0.3–157.4 g/m³ and –20 to 70°C, respectively. Their accuracy is ±5%, ±0.8 g/m³ and ±0.7°C. The data loggers were wrapped in metal gauze to prevent the bees covering the probes with propolis. The loggers were placed in the nectar stores and in the middle of the central brood comb of each hive (a piece of comb of the logger's size was cut out for this purpose). Although the loggers recorded the parameters as soon as they were embedded, we considered the data only after the brood temperature returned to 34.5 degrees, which suggested that the bees resumed normal activity. An empty hive without bees, brood or nectar comb served as a control for the effect of the hive itself on the parameters measured. After 4 days, the data loggers were removed and the data analysed. Cosinor analyses (Nelson et al. 1979) were performed to compare variations in AH and RH between colonies and between brood and nectar stores of each colony. For this, 15 consecutive 2-min interval measurements were averaged to obtain a point every half hour ($n=192$) over the 4 days monitored. Bonferroni correction (Dunn-Šidáček method, Sokal and Rohlf 1995) was applied when the parameters measured were compared for paired combinations of the three colonies (e.g. AH in the brood compared between colonies 1 and 2, between colonies 2 and 3 and between colonies 1 and 3). The level of significance adopted was 0.01.

Results

Control temperature varied from 3.7 to 30.7°C over the measurement period and was close to ambient conditions. Temperature in the nectar stores was higher and fluctuated to a lesser degree (14.6 to 38.1°C). Temperature in the brood area remained constant around 35°C (Fig. 1a). AH was low in the control hive and higher than ambient AH. In the nectar stores, it was higher on average and fluctuated widely. In the brood, AH was again higher, and still fluctuated, but within a narrower range compared to the nectar stores (Fig. 1b). RH in the nectar stores and brood area was higher than the control in two of the three colonies (Fig. 1c). Colony 3 had lower RH than the other colonies. In contrast to AH, RH was similar in the nectar stores and the brood area in two of the three hives. Colony 2 had a higher RH in the nectar stores (Fig. 1b and c). The inter-colonial variation in AH and RH patterns observed could not be explained on the basis of colony size. Cosinor analyses revealed significant differences in AH or RH between brood area and nectar stores of each colony (Table 1). There were also significant differences in AH between the brood areas of different colonies and between their nectar stores (Table 1). The same was true for RH (Table 1).

Control temperature and AH followed the same daily pattern, rising after sunrise to plateau during the day and decreasing progressively in the late afternoon until sunrise the next day (Fig. 2a and b). The same patterns were evident in the nectar stores, but with peak values being maintained for longer. In the brood, the trend for AH was opposite: AH increased in the late afternoon to drop the next morning (Fig. 2b). After a morning peak corresponding to dew formation, control RH decreased during the day due to the increase in temperature, then increased during the evening and night as temperature dropped (Fig. 2c). The pattern of in-hive variations in RH was similar to that

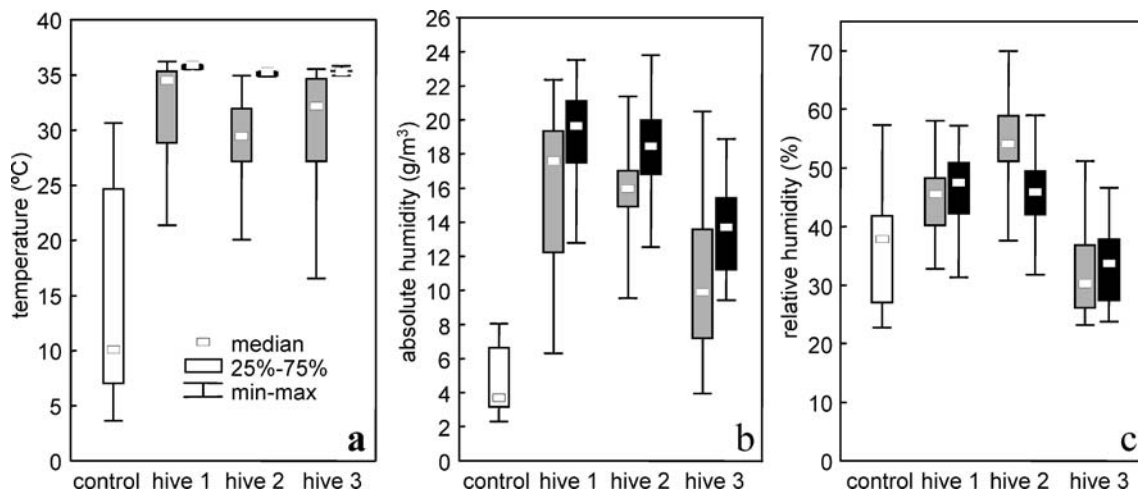


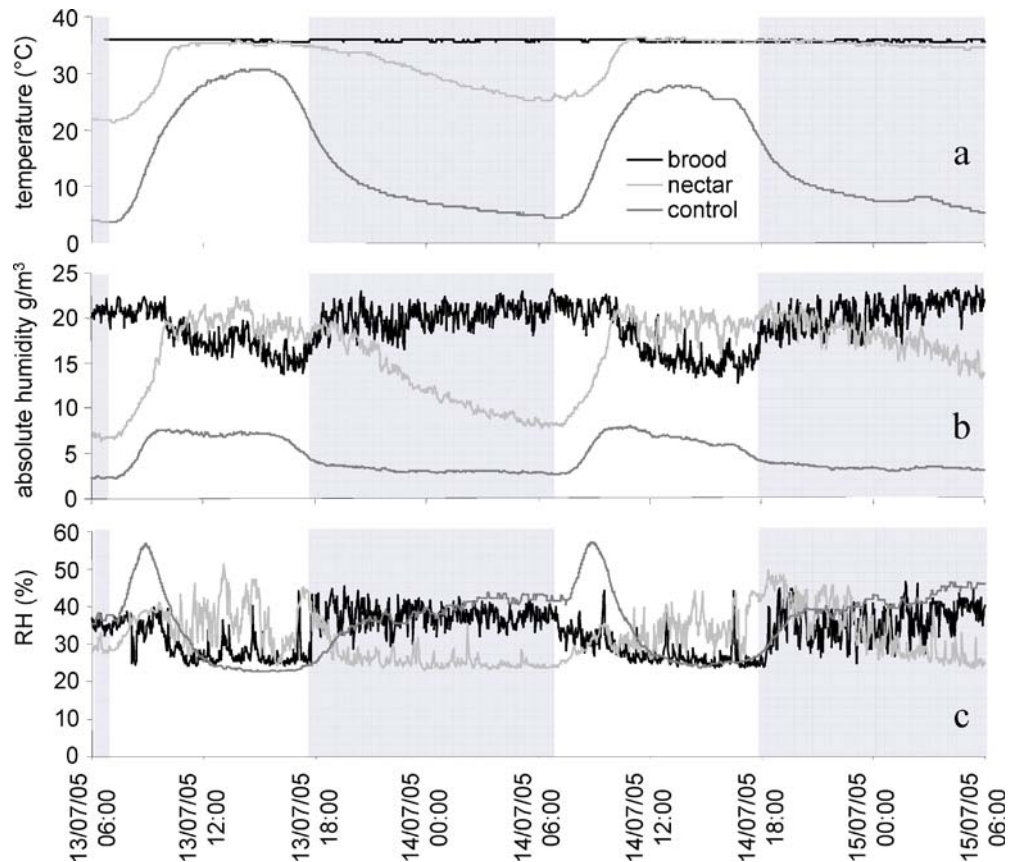
Fig. 1 Summary statistics for microclimatic parameters in three colonies measured over two consecutive days with similar weather. Data shown are: **a** temperature, **b** absolute humidity and **c** relative humidity in the nectar stores (grey bars) and in the brood area (black bars). Parameters measured in an empty hive are shown as a control (white bar)

Table 1 Cosinor analyses comparing AH between 1) brood areas of different colonies, 2) their nectar stores and 3) brood area and nectar stores of each colony

Area	Colony	Measurement	Brood colony 2	Brood colony 3	Nectar colony 1	Nectar colony 2	Nectar colony 3
Brood	1	AH	$F_{3,378}=17.8$ $p<0.0001$	$F_{3,378}=37.9$ $p<0.0001$	$F_{3,378}=58.9$ $p<0.0001$		
		RH	$F_{3,378}=23.3$ $p<0.0001$	$F_{3,378}=740.5$ $p<0.0001$	$F_{3,378}=40.5$ $p<0.0001$		
	2	AH		$F_{3,378}=542.5$ $p<0.0001$		$F_{3,378}=178.0$ $p<0.0001$	
		RH		$F_{3,378}=617.2$ $p<0.0001$		$F_{3,378}=23.5$ $p<0.0001$	
	3	AH					$F_{3,378}=42.4$ $p<0.0001$
		RH					$F_{3,378}=46.1$ $p<0.0001$
Nectar	1	AH				$F_{3,378}=19.6$ $p<0.0001$	$F_{3,378}=47.6$ $p<0.0001$
		RH				$F_{3,378}=35.7$ $p<0.0001$	$F_{3,378}=740.5$ $p<0.0001$
	2	AH					$F_{3,378}=29.4$ $p<0.0001$
		RH					$F_{3,378}=149.9$ $p<0.0001$

The same comparisons were made for RH. All *p* values are inferior to the Bonferroni corrected significance level (0.003) and, therefore, all differences are significant

Fig. 2 Variation in microclimatic parameters in a single colony over 2 consecutive days (data for 2 days only, out of 4, are presented for clarity). Data shown are **a** temperature, **b** absolute humidity and **c** relative humidity in the nectar stores and in brood area. Results obtained were similar for all three hives. Control parameters measured in an empty hive are also presented. *Shaded areas* represent night time



of AH, but the difference between brood area and nectar stores RH was of lower amplitude (Fig. 2c). RH rose during the day in the nectar stores, while it decreased in the brood area. At night, the trend was opposite (Fig. 2c).

Discussion

Large day–night fluctuations in temperature are characteristic of winters in Gauteng Province, South Africa. Minimum temperature was 3.5°C and maximum temperature was 31.3°C. Regardless of this high variation, *A. m. scutellata* bees were able to regulate brood temperatures with precision, confirming many previous studies (for a review, see literature in Moritz and Southwick 1992; Heinrich 1993).

Drought is another feature of the winters in this region. However, hive AH was always higher than control AH, indicating that it is not solely dependent on ambient humidity and that the humidity retention capacity of the hive does not explain the values measured. Although we found wide intercolonial variations, AH was always higher in the brood area where there is little nectar available as a source of water and a tendency for evaporation due to the high temperature maintained, but where there is also a high humidity requirement for optimal brood development (Doull 1976). This suggests that humidity in this area is maintained at a high level by the workers.

In contrast, AH in the nectar stores was lower, despite the high quantity of water evaporated during the honey ripening process (*A. greatheadii* var *davyana* nectar has a water content of 77%; Human and Nicolson, unpublished data). Decreasing humidity in these stores would allow the evaporation of nectar to honey and prevent microbial growth. The different AH measured in these areas and the lower amplitude variations of brood AH suggest that humidity is regulated, although not precisely. RH was more similar between the two areas monitored than AH. This is due to differences in temperature combined with the differences in AH.

The daily fluctuations of humidity in the brood area and nectar stores could be due to the honey ripening process. The fanning necessary to evacuate surplus water vapour generated by nectar concentration could decrease humidity level in the brood, given that these two areas share the same atmosphere, but not the same potential water vapour sources (nectar or transpiration). Active concentration of nectar by tongue lashing (Lindauer 1955) occurs just after unloading (Ribbands 1953) and stops together with foraging at dusk. At this time brood humidity could be restored to optimal levels. At night, the difference in humidity between these areas could be exacerbated by transpiration from a higher number of workers aggregated on the brood combs than on the nectar combs and by their insulating effect.

Figure 1 shows that all colonies regulated their brood temperature with similar efficiency. In contrast, there is no detectable optimum for AH. Temperature in beehives can be adjusted with precision because of the insulating

effect of the hive, honey stores (Lindauer 1955) and the bees' bodies (Starks and Gilley 1999). Furthermore, heat is produced by the bees themselves (Heinrich 1993) and transmitted to the brood by direct contact (Bujok et al. 2002). As a consequence, bees do not rely on an external heat source or on air movement to transmit heat. In addition, optimal temperatures are the same for all hive regions: high temperature favours optimal brood development and honey ripening.

In contrast, humidity modification necessitates water or nectar collection outside the hive and their evaporation, each step adding variability in the regulation mechanism. Limitations to humidity adjustment may also occur when no water is available (during droughts or at night) or when no water foragers are available (Wohlgemuth 1957). Furthermore, humidity optima differ in the brood area and nectar stores (high humidity is necessary for brood development, but a dry atmosphere favours nectar ripening). The difficulty of regulating humidity independently in each area might result in sub-optimal humidity levels. Humidity can also depend on trade-offs with other biophysical parameters, such as temperature or respiratory gases (e.g. Seeley 1974; Korb and Linsenmair 1998; Kleineidam and Roces 2000; Wohlgemuth 1957). For example, stale air has to be flushed out to allow clean air to enter the hive. Air at the optimal humidity will, thus, be expelled and replaced with air at ambient humidity. Humidity should, thus, be re-adjusted after each 'breathing' event (Southwick and Moritz 1987). This could explain the ragged aspect of the nectar store and brood humidity curves, in comparison to the control measurement (Fig. 2b and c).

Several facts have nurtured doubts about whether honeybees do regulate humidity in their hives or not (Ribbands 1953; Büdel 1960; Simpson 1961; Johansson and Johansson 1979; Willmer 1986). Monitoring devices used in the past were too large to differentiate between areas with different humidities. Furthermore, humidity may be only partially regulated due to the constraints and trade-offs mentioned above, and the absence of clear optimal humidity values could have hindered the recognition of a regulation mechanism. According to our hypothesis of humidity regulation in a hive, the optimal RH level is close to 40% (high plateau of brood RH in Fig. 2c).

Humidity levels measured in this study corresponded with those measured by others (Büdel 1960; Wohlgemuth 1957), but RH was below the optimum levels for brood development (>90%) identified by Doull (1976). Although microclimate in the cells is influenced by hive atmosphere, the humidity at the bottom of the cells, where brood develops, may be higher than our measured values. High moisture could be generated by the jelly (which has a high water content) in which larvae float and by water deposited in cells by workers and maintained through the insulation provided by dense worker cover (Doull 1976). Humidity in the brood area should then just be high enough to prevent desiccation of the cell atmosphere between the frequent visits of nurse bees (approximately every 9 min, calculated from Lindauer 1953).

We are currently investigating whether humidity is passively or actively regulated. Passive regulation could be based on transpiration of the hive's inhabitants and on the capacity effect of nectar (acting as a sink or source of water). Active regulation could be achieved by water collection and evaporation. Regulation of humidity would represent a sociophysiological mechanism that further contributes to the complex nest homeostasis of honeybees.

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