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Hygropreference and brood care in the honeybee (*Apis mellifera*)

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ABSTRACT

Terrestrial organisms need to limit evaporation from their bodies in order to maintain a homeostatic water balance. Owing to a large surface to volume ratio, arthropods are particularly susceptible to desiccation and have evolved behavioural and physiological mechanisms to conserve water. In social insects, water balance is also affected by the interactions between nestmates and by the architecture of the nest. For honeybees, humidity is particularly important for the brood because it affects the hatching success of eggs and because, unlike ants, honeybees cannot relocate their brood to parts of the nest with more favourable humidity. To advance the understanding of the water economy in honeybee nests, we investigated whether workers exhibit a hygropreference when exposed to a gradient of 24–90% relative humidity (RH) and whether the expression of this preference and their behaviour is affected by the presence of brood. The results show that young honeybee workers in the absence of brood exhibit a weak hygropreference for approximately 75% RH. When brood is present the expression of this preference is further weakened, suggesting that workers tend to the brood by distributing evenly in the gradient. In addition, fanning behaviour is shown to be triggered by an increase in humidity above the preferred level but not by a decrease. Our results suggest that humidity in honeybee colonies is actively controlled by workers.

1. Introduction

The large surface to volume ratio of arthropods accounts for their susceptibility to desiccation through cuticular and respiratory transpiration (Hadley, 1994). However, several aspects of arthropod physiology and behaviour serve to counteract this consequence of their small body size. For example, some tick species are able to absorb water vapour from unsaturated ambient air (Gaede and Knülle, 1997) and individuals of some Collembola species are able to locate microenvironments with low vapour pressure deficits and hence reduce evaporative water loss (Hayward et al., 2000). In social arthropods, water balance is not only dependent on the physiology and behaviour of individuals, but is also affected by the interactions between colony members and by their nest environment. For instance, the nest architecture of some social insect species ensures that suitable microclimatic conditions occur in the nest (Sherba, 1959; Frouz, 2000; Kleineidam and Roces, 2000) thus making it possible for the workers to select certain areas of the nest for certain activities. Humidity based decision-making has been shown in leaf-cutting ants of the genus *Atta* (Roces and Kleineidam, 2000; Ribeiro and Navas, 2006), four species of fire ants from the genus *Solenopsis* (Potts et al., 1984), the wood ant *Formica rufa* (North, 1991), the meat ant *Iridomyrmex* sp. and the Argentine ant, *Linepithema humile* (Walters and Mackay, 2003). These studies have shown that ants prefer humidities of greater than 90% RH, and *Atta sexdens* and *Solenopsis* sp. relocate their fungus garden or brood to locations where the growing conditions are optimal.

Humidity is also an important microclimatic variable for honeybees (*Apis mellifera* L.) since their eggs require a relative humidity (RH) of above 55% to hatch successfully, with the highest survival between 90 and 95% RH (Doull, 1976). High humidity would also benefit brood development indirectly since the reproductive success of *Varroa* parasitic mites decreases with increasing humidity (Kraus and Velthuis, 1997). However, adult honeybee survival has been shown to decrease with increasing humidity (Woodrow, 1935) and the percentage of brood mortality caused by chalkbrood (*Ascosphaera apis*) was shown to increase by 7% when RH was increased from 68 to 87% (Flores et al., 1996). Unlike ants, honeybees are unable to relocate their brood to the part of the nest most suitable for development. Indeed, eggs remain in the cell in which the queen laid them and develop in this same cell until emergence of the adult. Honeybee workers would therefore need to regulate humidity to optimal levels in the brood nest to favour brood development. There are a number of behaviours in the repertoire of honeybee workers that may be

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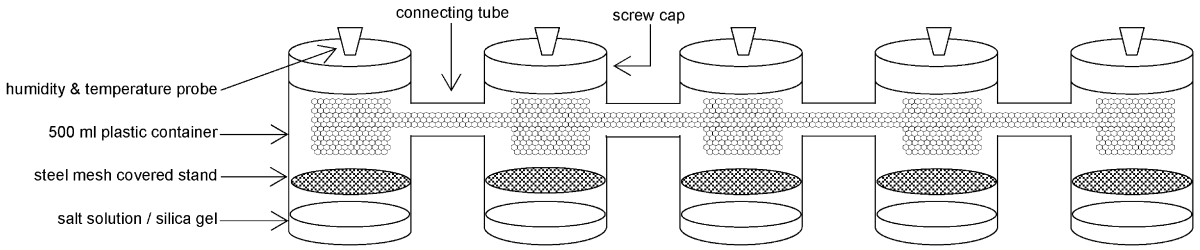

Chamber	24%	40%	55%	75%	90%
RH Gradient	Silica Gel 24 ± 3.4%	$MgCl_2$ 40 ± 6.0%	$Mg(NO_3)_2$ 57 ± 4.8%	NaCl 76 ± 3.8%	$K_2Cr_2O_7$ 90 ± 2.0%
	Silica Gel 24 ± 8.8%	NaI 39 ± 1.4%	$Na_2Cr_2O_7$ 54 ± 1.5%	$NaNO_3$ 72 ± 1.8%	K_2SO_4 89 ± 2.1%

Chamber	55%
RH Constant	$Mg(NO_3)_2$ 53 ± 3.4

Fig. 1. Five linearly arranged humidity chambers used to maintain two different sets of humidities; one setup with a gradient of 24–90% RH and another with 55% RH in all chambers. Two sets of salts were used in the 24–90% RH chambers to prevent bias caused by preference for a particular salt. Values indicated are the measured % RH (±S.D.) in each chamber.

used to alter nest humidity. Ventilation of the hive through fanning behaviour has been implicated in thermoregulation (Hazelhoff, 1954; Lindauer, 1961; Lensky, 1964) and carbon dioxide regulation (Hazelhoff, 1941; Seeley, 1974; Southwick and Moritz, 1987), but is also expected to influence nest humidity. Furthermore, nectar dehydration (Reinhardt, 1939) and water collection and spreading in the nest (e.g. Lindauer, 1954; Kühnholtz and Seeley, 1997) could be used to increase relative humidity.

Electrophysiological studies have demonstrated that coeloca-pitular sensilla located on the antennae of honeybees are stimulated by changes in humidity (Lacher, 1964; Yokohari et al., 1982). This shows that honeybees can detect fluctuations in humidity, but it is not known whether they alter their behaviour according to such stimuli or change the intensity or frequency of their behaviour. This study investigates whether honeybee workers exhibit a hygropreference when exposed to a humidity gradient of 24–90% RH, and whether the expression of this preference is dependent on the presence of brood. We also determined how different humidities affect fanning and general activity levels. We hypothesised that in the absence of brood honeybee workers would detect differences in humidity in different chambers and relocate to decrease their evaporative water loss. In contrast, in the presence of brood, we expected them to respond to suboptimal RH by fanning or by altering their activity levels in an attempt to improve developmental conditions.

2. Methods

2.1. Experimental animals and rearing conditions

We used honeybee (*Apis mellifera scutellata*) workers from eight different colonies housed in the University of Pretoria apiary. A frame of brood was removed from each colony and placed into a Perspex box in an incubator at 60% RH and 35 °C, which is the optimal temperature for brood development. Within 24 h of emergence, the workers were collected, placed in hoarding cages (dimensions: 90 cm × 100 cm × 70 cm) with *ad libitum* food (a sucrose, honey and pollen mixture) and water and returned to the incubator. Due to the age polyethism that is partially responsible for differentiation of tasks within a colony, hygropreference of social insects could vary according to age. Workers of 3 and 6 days old are normally involved in cell cleaning and brood tending respectively (Lindauer, 1952) and these age groups may respond differently to a humidity gradient. Freshly emerged workers from a single colony were therefore maintained in hoarding cages for 3 or 6 days and then tested for hygropreference ($N = 8$ colonies tested per age) to determine whether age influences worker behaviour in a humidity gradient.

2.2. Hygropreference of workers without brood

Experimental trials were conducted in a dark climate-controlled room which was heated by two heater fans (Tempadait, Fan Heater, Johannesburg) regulated by a thermistor (A419, Johnson Controls, Milwaukee, USA). Although insects are known to alter their hygropreference based on temperature (Haywood et al., 2001; Prange and Hamilton, 1992), we tested hygropreference of honeybee workers at a single temperature of 34.5 ± 0.5 °C since this is the temperature at which brood is reared and the temperature at which the experimental workers would be found within the nest.

Gradients of RH (as in Roces and Kleineidam, 2000; Walters and Mackay, 2003) were established in a set of five linearly arranged 500 ml plastic screw cap jars, interconnected with transparent tubing (length 5 cm, diameter 2 cm) and containing mesh covered stands to prevent workers from contacting the salt solution or silica gel (Fig. 1). Pieces of freshly drawn comb (10 by 20 cells) were placed in each container and connected by a strip of wax (length 9 cm, height 2 cm) placed in each connecting tube. This created a continuous vertical substrate for movement of bees from one chamber to another. The RH gradient was generated using silica gel (24% RH) and the following set of saturated salt solutions: 33% RH, $MgCl_2$; 51% RH, $Mg(NO_3)_2$; 76% RH, NaCl; 97% RH, $K_2Cr_2O_7$ (Winston and Bates, 1960). Although no volatiles are expected from the salts, they were substituted by the following combination after completion of half the trials in order to prevent bias caused by preference for a particular salt: 34% RH, NaI; 51% RH, $Na_2Cr_2O_7$; 71% RH, $NaNO_3$; 96% RH, K_2SO_4. Since the chambers were interconnected, gas exchange might occur between them and alter the expected humidity. To account for this effect, humidity was recorded in each chamber with a probe (SHT75, Sensirion, Zürich, Switzerland, ±1.8% RH, set to record every 2 s): no overlap of RH between chambers was recorded. Based on the measured values, chambers were termed the 24%, 40%, 55%, 75% and 90% RH chambers respectively (Fig. 1).

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Before each trial, 100 workers were placed in a refrigerator and cooled down to facilitate handling. Twenty individuals were then introduced into each chamber and for each consecutive trial the sequence of introduction was alternated between the ends of the RH gradient. Workers were allowed to acclimate for the first hour which also ensured that the RH level stabilised after the disturbance created by opening the chambers. Observations were carried out every 30 min for the subsequent 3.5 h after which the distribution of workers stabilised. In order to determine the hygropreference of honeybees the number of workers in each chamber was recorded. If a worker was located in the tube between chambers, the direction of its head was used to indicate its preference.

The distribution of workers in linearly arranged chambers can be influenced by uncontrolled factors with individuals aggregating non-randomly in chambers at either end of the array. The occurrence of this bias can be excluded if workers distribute themselves randomly between chambers with identical humidity. We therefore measured the distribution of workers in a setup where a humidity of 55% RH (which frequently occurs in honeybee nests, Human et al., 2006) was maintained in all chambers using a $Mg(NO_3)_2$ solution ($N = 5$ colonies).

2.3. Hygropreference of workers in the presence of brood

It is possible that, like fire ants which fail to show a clear hydrokinetic response in the absence of brood (Potts et al., 1984), the behaviour of honeybees in a humidity gradient could be altered by the availability of brood. Since 6-day-old workers are more likely to perform tasks related to brood care (Lindauer, 1952), we did not test the hygropreference of 3-day-old workers in the presence of brood. We monitored the behaviour of 6-day-old workers exposed to eggs and larvae. Differences in behaviour between experiments in which workers were exposed to eggs or larvae was expected if workers respond to different desiccation rates of these brood types or if they display preference for one of these brood types based on age polyethism (Ribbands, 1953, p. 301). Trials ($N = 4$ colonies) were conducted in which ten eggs were grafted into the comb within each chamber and another set of trials ($N = 4$ colonies) using ten 1st to 3rd instar larvae. Grafting enabled selection of brood from the relevant colony and standardization of the amount and developmental stage of the brood that was placed into the comb in each chamber. After grafting, workers were introduced into the chambers and after 1 h of acclimation the distribution of workers was recorded as described above. At the end of each trial the brood was removed, and pieces of wax were changed every three to four trials.

2.4. The effect of humidity on fanning behaviour and worker mobility

We monitored the number of fanning workers per chamber and the number of actively mobile workers per chamber. Since we transferred workers into an artificial setup where few tasks can be performed some behaviours might not be expressed. We therefore monitored workers' mobility as a proxy for general activity level. Mobility was determined by counting the number of workers that were moving for longer than 2 s around the chamber or across the surface of the comb. Observations were made using a low power headlamp. Fanning and mobility observations were recorded as a percentage of the total number of workers in a particular chamber.

2.5. Statistical analysis

The mean percentages of live workers, fanning workers and mobile workers per chamber were calculated for all observations during the 3.5 h experimental period and these values were used for analysis. The mean mortality (±S.D.) was 1.3 ± 2.86% at the end of all replicates and all replicates with a mortality exceeding 16% were excluded from analysis ($N = 4$). Some colonies were tested more than once for a particular age and the data were averaged for each chamber and constituted one replicate. The effect of humidity on the distribution and behaviour of workers in the five chambers was determined using a Friedman ANOVA. Pairwise comparisons between chambers were calculated using a Wilcoxon matched pairs test (with Bonferroni correction). In order to determine whether age affects hygropreference, the mean number of workers per humidity (i.e. chamber) was calculated across the eight replicates for 3-day-old individuals and likewise for 6-day-old individuals. These mean distributions were compared using a Mann–Whitney U test in order to determine the effect of age on hygropreference. The same test was used to compare the mean distribution of workers in the presence and absence of brood to determine the effect of the availability of brood on hygropreference. The software STATISTICA version 7.1 (Statsoft Inc., 1996) was used for statistical analysis.

3. Results

3.1. Hygropreference of workers without brood

Data from the linear array of humidity chambers (24–90% RH) showed that the mean number of workers in each chamber did not differ significantly between ages 3 and 6 days (Mann–Whitney test: $U = 9.0$, $N = 5$, N.S.). The data for 3 and 6 days were therefore pooled and showed a non-random distribution of workers in the chambers with different humidities (Friedman ANOVA $\chi^2 = 28.4$, d.f. = 4, $p < 0.01$; Fig. 2). In the absence of brood, the number of workers in the 75% RH chamber was significantly higher than in all other chambers (Wilcoxon matched pair test: $Z < 15.1$, $N = 16$, $p < 0.05$) and the number in the 90% RH chamber was significantly lower than all others (Wilcoxon matched pair test: $Z < 16$, $N = 16$, $p < 0.05$) except for the 55% RH chamber (Wilcoxon matched pair test: $Z = 27$, $N = 16$, N.S., Fig. 2).

The preference of the workers was not dependent on the position of the chamber in the linear setup since the distribution of workers between the five 55% RH chambers was not significantly different from random (Friedman ANOVA $\chi^2 = 8.16$, d.f. = 4, N.S.).

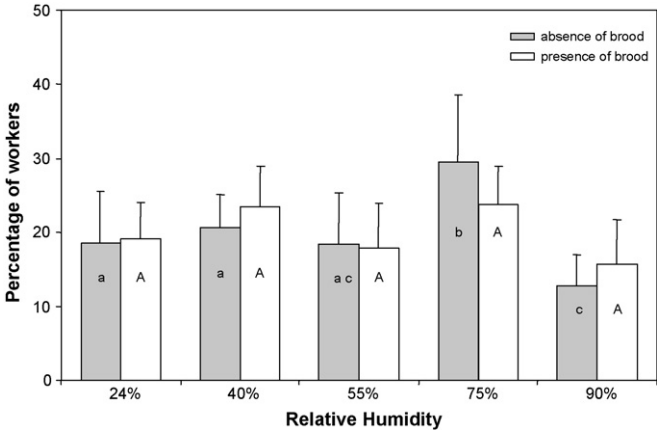

Fig. 2. The mean distribution of *A. mellifera scutellata* workers during the 3.5 h of exposure to a humidity gradient of 24–90% RH in the absence (3- and 6-day-old workers) of brood and the presence (6-day-old workers) of brood. Means (±S.D.) for each humidity are presented ($N = 16$) and letters indicate significant differences at $p < 0.05$ (Wilcoxon matched pairs test). No significant differences were found between the number of workers in the different humidities in the presence of brood.

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3.2. Hygropreference of workers in the presence of brood

The distribution of workers in the presence of eggs did not differ significantly from that of workers in the presence of larvae (Mann–Whitney test: $U = 11.00$, $N = 5$, N.S.). The data for eggs and larvae were therefore pooled and showed that workers were unevenly distributed among the chambers (Friedman ANOVA $\chi^2 = 20.6$, d.f. = 4, $p < 0.05$). However, a pairwise comparison of the chambers yielded no significant differences (Wilcoxon matched pair test: $Z > 3$, $N = 8$, N.S., Fig. 2).

3.3. The effect of humidity on fanning behaviour and worker mobility

The distribution of fanning workers was not dependent on the position of the chamber in the linear setup since their number was not significantly different between the five 55% RH chambers (Friedman ANOVA $\chi^2 = 5.17$, d.f. = 4, N.S). This number was consistently low in all chambers with a mean (±S.D.) of 0.3 ± 0.1 workers fanning per chamber during an observation time.

The number of fanning workers in the 24% to 90% RH gradient was strongly influenced by the humidity in the chambers (Friedman ANOVA $\chi^2 = 53.03$, d.f. = 4, $p < 0.01$, Fig. 3) and showed a steady increase with increasing humidity. The mean (±S.D.) number of fanning workers in each chamber during an observation was 0.1 ± 0.04, 0.1 ± 0.03, 0.4 ± 0.24, 2.9 ± 0.77 and 3.1 ± 0.91, from low to high humidity chambers respectively. When the chambers were tested pairwise, all chambers differed significantly in the number of fanning workers (Wilcoxon matched pair test: $Z > 2.07$, $N = 16$, $p < 0.05$) except for the 24% RH versus 40% RH chambers (Wilcoxon matched pair test: $Z = 1.34$, $N = 16$, N.S., Fig. 3). In the presence of brood, a Friedman ANOVA showed an uneven distribution of fanners amongst the chambers ($\chi^2 = 26.29$, d.f. = 4, $p < 0.01$, Fig. 3). However, a combination of pairwise tests showed that the 24, 40 and 55% RH chambers contained a significantly lower number of fanners than the 75 and 90% RH chambers (Wilcoxon matched pair test: $Z > 2.52$, $N = 8$, $p < 0.05$). The 24, 40 and 55% RH chambers and the 75 and 90% RH chambers did not contain significantly different numbers of fanners (Wilcoxon matched pair test: $Z < 2.24$, $N = 8$, N.S.). The number of fanning workers in the presence of brood was higher in the 75 and 90% RH chambers compared to the number of fanners in the absence of brood, but this difference was not significant (Mann–Whitney test: $U = 756.5$, $N = 40$, N.S.).

The number of mobile workers differed significantly between humidities in both the absence (Friedman ANOVA $\chi^2 = 26.8$, d.f. = 4, $p < 0.01$, Fig. 4) and presence of brood (Friedman ANOVA $\chi^2 = 25.7$, d.f. = 4, $p < 0.01$) and showed a consistent decrease with increasing humidity. A significantly smaller number of workers was observed to be mobile when in the presence of brood (mean ± S.D., 3.9 ± 0.5) compared to the absence of brood (5.1 ± 0.9, Mann–Whitney test: $U = 508.0$, $N = 40$, $p < 0.01$, Fig. 4). The number of mobile workers was not dependent on the position of the chamber in the linear setup since the distribution of workers between the five 55% RH chambers was not significantly different from random (Friedman ANOVA $\chi^2 = 3.68$, d.f. = 4, N.S.).

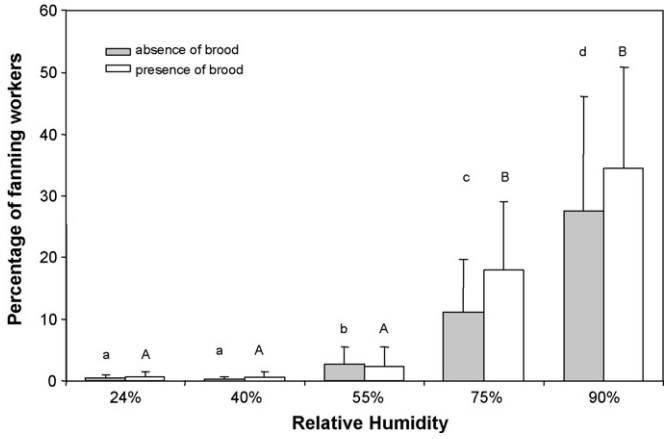

Fig. 3. Percentage of fanning workers given as mean (±S.D.) per chamber in a RH gradient (24–90% RH). Letters a–d indicate significant differences in the presence (6-day-old workers) of brood and letters A–B indicate differences when in the absence (3- and 6-day-old workers) of brood ($p < 0.05$, Wilcoxon matched pairs test).

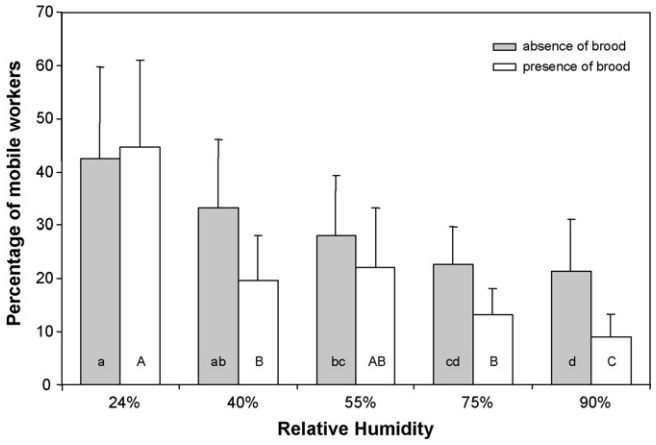

Fig. 4. Mean percentage (±S.D.) of mobile workers in a humidity gradient of 24–90% RH when in the absence (3- and 6-day-old workers) of brood and in the presence (6-day-old workers) of brood. Letters a–d indicate significance at $p < 0.05$ in absence of brood and letters A–C in the presence of brood (Wilcoxon matched pairs test).

4. Discussion

The results of this study show that young honeybee workers in the absence of brood exhibit a hygropreference for a humidity of approximately 75% RH at 34.5 °C. When brood was present, this preference was expressed to a lesser degree and worker fanning and mobility levels were altered.

Control experiments with uniform RH showed that neither the distribution, fanning behaviour nor mobility of honeybee workers was dependent on the position of the chamber in the linear setup. All the differences in behaviour we observed were therefore due to differences in humidity. In the absence of brood, i.e. without the availability of tasks related to brood care that could lead to a differentiation of behaviour based on age polyethism, workers of 3 and 6 days of age showed the same humidity preference. This is in spite of the fact that these groups might have performed different tasks prior to their placement in the experimental setup. It is possible that, based on differences in humidity of the different nest regions (Human et al., 2006) where workers are active, individuals would show different hygropreferences. Workers caring for brood (6-day-old) and brood cell cleaners (3-day-old) could have a preference for higher humidity compared to workers active in the drier region of nectar stores (Human et al., 2006). In addition, we detected no difference in the behaviour of workers that were exposed to eggs or larvae, suggesting a similar sensitivity of these brood types to desiccation and no preference of 6-day-old workers for tending either of them.

Our results diverge from similar studies conducted on some ground dwelling ant species in that honeybee workers show a preference for a humidity of approximately 75% RH and not for an extremely high humidity (90% RH, Walters and Mackay, 2003; Roces and Kleineidam, 2000; North, 1991; Potts et al., 1984). The amount of water required to saturate the brood nest at 35 °C is

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approximately 1.1 ml and this volume could evaporate from the abundant sources of moisture (e.g. royal jelly, respiration of nest inhabitants, nectar) and saturate the nest's atmosphere with water vapour. The fact that such high humidity is not measured in hives (Human et al., 2006) and that a preference for a lower humidity level was detected in our study suggests that it could be adaptive for honeybees to actively decrease humidity in the nest to a preferred level. Avoidance of high humidities could contribute to an increase in adult longevity and decreased microbial development. The results of this study suggest that a humidity of approximately 75% RH (higher than 55% and lower than 90% RH) is an optimal value in the brood nest. This value is higher than that measured by Human et al. (2006) in hives in the field during the dry South African winter, but comparable to other measurements in field colonies conducted simultaneously with the present study, in spring and summer (Ellis MB, unpublished data). This discrepancy could thus be due to different measuring conditions (laboratory versus field) or to seasonal or intercolonial variation in humidity regulation or preference.

These observations also differ from other similar studies in that the response of honeybee workers to a humidity gradient was weak, with a mean of 30% of the honeybee workers selecting the 75% RH chamber. It is likely that some characteristics of the experimental design (e.g. discontinuous gradients, absence of brood in the connecting tubes) prevent the movement of bees between chambers and the expression of a strong preference. However, using similar designs, strong hygropreference was shown for many ant species with most of the workers gathering in the chamber with the highest humidity (North, 1991; Walters and Mackay, 2003). Rather than an experimental artefact, the weak preference observed could therefore correspond to a real biological phenomenon if honeybees can actively regulate humidity in their nest. Indeed, such regulation mechanisms would necessitate the dispersal of workers throughout a humidity gradient enabling them to actively counteract sub-optimal conditions where they occur. This idea is supported by the fact that fanning was consistently low below 55% RH and increased with relative humidity, indicating that this behaviour is aimed at removing humid air from the hive in a natural situation. Mobility increased with increasing vapour pressure deficit, which could also result from the workers trying to regulate humidity by other means than fanning, such as water collection and spreading.

In some ant species hygropreference is dependent on the presence of brood or fungal gardens (Potts et al., 1984; Roces and Kleineidam, 2000). In contrast, in the honeybee, the expression of hygropreference was further weakened when workers were exposed to brood. Although a Friedman ANOVA detected a non-random distribution of workers in our chambers with different RH, a pairwise test showed that these variations were not sufficient to result in significant differences between the numbers of workers in each chamber. We hypothesise that workers dispersed throughout the experimental setup in an attempt to regulate humidity for the desiccation-sensitive brood that cannot be moved between chambers and that this resulted in an almost even distribution. This idea is supported by our observations that fanning activity by workers was higher in the presence of brood, albeit not significantly so. This suggests that the presence of brood further stimulates the workers to counteract adverse conditions by adjusting the humidity to optimal levels. In addition, significantly fewer workers were mobile in the presence of brood. This is likely to reflect the fact that the addition of brood resulted in some workers settling on the comb to care for the larvae or eggs. It is worth noting that the occurrence of fanning and mobile workers in the absence of brood shows that the presence of brood is not essential for workers to attempt to adjust adverse humidity conditions: the presence of other workers might be enough to trigger these behaviours.

In order to maintain stable nest homeostasis, honeybee workers are able to regulate various microclimatic parameters within the hive. For instance, when the brood nest temperature increases by 3 °C from 34 to 37 °C, the number of fanning workers increases ten-fold (Lensky, 1964) and an increase in the CO_2 level in hives can cause a thirty-fold increase in fanning workers (Seeley, 1974). Under our experimental conditions when all other factors are held constant, the number of fanning workers increases 10 times when humidity increases from 55 to 90% RH. This shows that a single behaviour, i.e. fanning, can affect several microclimatic parameters. In the same way, there are various behaviours involved in thermoregulation within the hive, such as water spreading and tongue lashing, which can also affect humidity. This can lead to the occurrence of tradeoffs in the adjustment of each optimum, as is evident in termite and ant nests (Korb and Linsenmair, 1999; Kleineidam and Roces, 2000) and can prevent honeybees from regulating some of these factors optimally (Human et al., 2006).

Several other factors are likely to influence humidity within the hive. Relative humidity is dependent on temperature and the thermoregulation of the colony is therefore directly linked to the relative humidity within the nest. Larval cocoons are hygroscopic and can provide a buffering effect on humidity fluctuations (Chauvin et al., 1979). Since larval cocoons accumulate in the cells in which the brood develops (Hepburn and Kurstjens, 1988), the physical properties of the comb can buffer humidity fluctuations (Ellis MB, unpublished data). Water also evaporates from the nectar stores; however this source is seasonal and dependent on floral availability and quality. Evaporative water losses from the bodies of the nest inhabitants can also increase nest humidity and the phenomenon of brood rearing in the winter cluster has thus been described as a strategy to reduce the water content of overwintering colonies (Omholt, 1987). Since so many factors influence humidity, detailed studies of their role and interactions will be necessary to understand the water economy of a honeybee hive. Our results provide the first demonstration that fanning can be triggered by an increase in humidity, suggesting that humidity is yet another microclimatic variable that is actively controlled by honeybee colonies. We suggest that the ability of honeybee workers to regulate this parameter weakens the expression of their preference for a particular RH.

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