

CHAPTER 5

Do honeybees, *Apis mellifera scutellata*, eliminate excess water from the dilute nectar of *Aloe greatheadii* var *davyana* before returning to the hive?

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Abstract

Aloe greatheadii var *davyana* flowers during the dry winter months across the northern summer rainfall areas of South Africa. Nectar is continuously available throughout the day, at an average concentration of about 20% w/w. The crop contents of nectar foragers were sampled at two sites in Gauteng Province to determine whether changes in nectar concentration occurred after collection and before unloading in the hive. Possibly because this nectar is so dilute for honeybees, relatively small volumes, $10.8 \pm 8.8 \mu\text{l}$ at Roodeplaat Nature Reserve and $15.8 \pm 6.6 \mu\text{l}$ at Rust de Winter, were transported back to the hive. We observed a significant increase in nectar concentration, accompanied by a decrease in nectar volume, between the flowers and the hive. At Roodeplaat Nature Reserve the nectar concentration increased from 21.3% in the flowers to 32.0% in the crops of honeybees captured at the flowers and at Rust de Winter from 21.8 to 35.6%. We observed a further increase in bees captured at the hive entrance. This dramatic increase in concentration of the crop contents between the flowers and the hive is unexpected in view of the common assumption that nectar is either unchanged or slightly diluted during transport.

Introduction

Aloe greatheadii var *davyana* is an extremely important indigenous plant for South African beekeepers, with a widespread distribution across the northern summer rainfall regions (Van Wyk & Smith, 1996; Glen & Hardy, 2000; Williams, 2002). This aloe flowers in mid-winter, from June to August, at a time when little else is flowering. It is common for beekeepers to move their hives to the “aloe fields” north of Pretoria during winter. The strong pollen and nectar flow is used for honey production, to rear queens, and to build up colonies and increase colony numbers by division (Jackson, 1979; Williams, 2002). The pollen of *A. greatheadii* var *davyana* is an excellent food source, having a very high protein content (Chapter 1).

The nectar of *A. greatheadii* var *davyana* is available to floral visitors throughout the day, with an average standing crop of $14.7 \pm 7.1 \mu\text{l}$ (volume) and $18.6 \pm 2.7\%$ (concentration) (Chapter 4). Although dilute for honeybees, this nectar is more concentrated than that of other *Aloe* species. In general, *Aloe* species produce copious quantities of very dilute (10-15%), hexose-dominant nectars (Van Wyk et al., 1993; Nicolson, 2002). For example, *A. ferox* produces large volumes (180 μl per flower) with a low concentration (12.5%) (Hoffman, 1988), while the concentration of *A. castanea* nectar remains below 10% throughout the day (Nicolson & Nepi, 2005).

Dilute nectar is associated with bird pollinators (Nicolson, 2002) while honeybees generally prefer nectar with 30-50% sugar (Southwick & Pimentel, 1981). However, honeybees have been shown to collect nectar over a much wider range of concentration (e.g. 15-65% w/w, Visscher & Seeley, 1982). Dilute nectars do not necessarily deter bees: when they need to cool the hive by evaporation they will collect nectar with lower concentrations (Eisikowitch & Masad, 1982; Ohguchi & Aoki, 1983). Lindauer (1955) observed honeybees collecting both water and nectar on a daily basis from spring until autumn. The water need of a colony is affected by the quantity of nectar; during a good nectar flow less water is needed (Lindauer, 1955). During winter, when the temperature is lower and the air is drier, the water needs of honeybee colonies may increase (Johansson & Johansson, 1978). In addition to providing energy, the nectar of *A. greatheadii* var *davyana* may serve as a source of moisture to honeybees during the dry winter months.

Utilising dilute nectar as a food source poses a problem to bees, by increasing the amount of excess water that needs to be eliminated during the ripening of honey. Extensive evaporation will be necessary with utilization of the dilute nectar of *A. greatheadii* var *davyana*. This process could begin before unloading of the crop contents in the hive. However, Park (1932) observed no increase in sugar concentration in the honeybee crop between the nectar source and the hive entrance when bees were collecting nectars of about 30%. Since then it has been generally accepted that the concentration of nectar in the forager's crop is an accurate indication of the nectar concentration of the flowers it has been visiting, and this has in fact been used as a method of sampling nectar (see Roubik & Buchmann, 1984; Roubik et al., 1995). In addition Oertel et al. (1951) observed a dilution of crop contents after the consumption of experimental syrups by honeybees and attributed it to the addition of glandular secretions and rapid inversion of sucrose.

Park (1932) trained field bees to feed at a feeder and starved the bees for at least 1 h before conducting his experiments. Oertel et al. (1951) placed bees in cages and starved them for 1.5 h before feeding them experimental syrups of varying concentrations. These periods of time were sufficient to ensure that the bees' crops were empty. Our study was conducted in natural field sites with dense stands of *A. greatheadii* var *davyana*, and bees were allowed to forage normally on the dilute hexose-rich (only 2% sucrose) nectar (Van Wyk et al., 1993). Since *A. greatheadii* var *davyana* flowers when nothing else is flowering, there could only be one source of the nectar brought back to the hive. Honeybees were captured on flowers and at the hive entrance to investigate whether nectar concentration in the crop changed between foraging at flowers and arrival at the hive.

Methods

Prior to the onset of flowering, six honeybee (*Apis mellifera scutellata* L) hives were moved to Roodeplaat Nature Reserve (795 ha) (28° 39'E, 25° 66'S) in Gauteng Province as part of a broader study of the interactions between the bees and the aloes. Observations on bee foraging were conducted at Roodeplaat Nature Reserve and also at Rust de Winter (28° 23'E, 25° 12'S). There were approximately 300 beehives at Rust de

Winter. Both areas have dense populations of *A. greatheadii* var *davyana*, especially Rust de Winter, and the flower patches and hives were less than 300 m apart.

We captured 30 bees leaving hives and another 30 bees returning to hives at each site, and an additional 30 swarming bees at Rust de Winter. We placed the bees on ice and weighed them in the laboratory with a Sartorius micro scale (Gottingen, Germany) to determine the mass of the nectar loads.

The crop contents of nectar foragers were sampled between 09.00 and 13.00 h on two consecutive days (11 - 12 July 2004). We sampled residual nectar from flowers that were visited and the crop contents of three categories of bees: foragers captured at the flowers, foragers arriving at the hive entrance, and swarming bees. On each day:

1. We captured 50 honeybees at flowers after they had collected nectar for >20 s (determined to be the average honeybee visit duration, pers. obs.), expressed and measured their crop contents and simultaneously removed the flowers that had been visited to measure the volume and concentration of residual nectar.
2. We blocked the hive entrances between 10.00 and 11.00 h and captured 50 returning foraging bees in Ziploc plastic bags. The bags were placed on ice to facilitate handling of the bees.
3. At Rust de Winter we also managed to capture and express the crop contents of 50 swarming bees.

Volumes of residual nectar were determined from column length in disposable haematocrit tubes (length 75 mm/75 μ l) and the concentrations measured as % w/w sucrose equivalents with a pocket refractometer (0-50%, Bellingham & Stanley Ltd, Tunbridge Wells, UK). Crop contents of all bees were extracted within 10 min of capture. Bees were induced to regurgitate by pressing the thorax dorsoventrally (Roubik & Buchmann, 1984) and the crop contents were then collected from the mouthparts in haematocrit tubes. The volume and concentration of the crop contents were measured as for nectar. Because the crop contents of swarming bees were extremely viscous, only concentration could be measured, using a high range pocket refractometer (40-85%, Bellingham & Stanley Ltd, Tunbridge Wells, UK).

Statistical analysis

Data for bee weights and for bee crop contents (volume and concentration) did not meet the assumptions for parametric statistics; variances were not homogeneous and data did not conform to a normal distribution. Due to significant differences between the two sites we were unable to pool the data and each site was analysed separately. Variation in volume and concentration of the crop contents of honeybees captured at the flowers and at the hive entrance, and nectar remaining in the flowers, were therefore assessed with Kruskal-Wallis ANOVA (Zar, 1984) and the level of significance was $P < 0.05$. Mann-Whitney U-tests were used for paired comparisons, including crop contents of swarming bees captured at Rust de Winter. Bonferroni corrections were applied for all paired combinations.

Analyses were performed with the program Statistica 6.0 (1984-2004). Values are given throughout as means \pm SD.

Results

As a result of low ambient temperatures during the winter flowering season, bee foraging only started at 09.00 h in the mornings and stopped after 16.00 h, amounting to a working period of only seven hours. Flowers opened throughout the day, so both pollen and nectar were continuously available (Chapter 4). Honeybees appeared to collect only nectar or pollen, not both. Bees obtained nectar by partially or completely entering the tubular flowers.

The average mass of returning foragers at Roodeplaat Nature Reserve was 73.6 ± 4.6 mg and that of foragers leaving the hive was 61.7 ± 2.8 mg, resulting in a mean weight of 11.6 ± 5.0 mg for nectar loads which may include small volumes at departure. At Rust de Winter the average weight of returning foragers was 77.7 ± 8.2 mg, and that of foragers leaving the hive was 62.9 ± 2.9 mg, resulting in a mean weight of 13.9 ± 7.9 mg for nectar loads. The average nectar loads include any initial honey consumed prior to leaving the hive. Swarming bees weighed on average 100.6 ± 10.6 mg resulting in a load mass of 37.7 ± 9.2 mg and a calculated volume of $27.9 \mu\text{l}$ for nectar loads (based on the density of the 71% sugar solution in their crops; Fig. 1B). There was a significant

difference between the body masses of foragers captured at Roodeplaat Nature Reserve and Rust de Winter ($U = 325.0$, $P > 0.05$).

Table 1. Results of paired comparisons of means for residual nectar and crop contents of bees (Mann-Whitney U-test). Adjusted P values after Bonferroni corrections are $P < 0.025$ for Roodeplaat and $P < 0.017$ for Rust de Winter. Significance is shown by italics.

Roodeplaat		Crop contents of bees at flowers	Crop contents of returning foragers
Residual nectar	Volume	<i>$U = 550.00$, $P < 0.0001$</i>	<i>$U = 505.50$, $P < 0.0001$</i>
	Concentration	<i>$U = 6.00$, $P < 0.0001$</i>	<i>$U = 5.00$, $P < 0.0001$</i>
Crops contents of bees at flowers	Volume		$U = 980.50$, $P > 0.05$
	Concentration		<i>$U = 401.50$, $P < 0.0001$</i>
Rust de Winter			
Residual nectar	Volume	$U = 1014.50$, $P = 0.105$	<i>$U = 651.00$, $P < 0.0001$</i>
	Concentration	<i>$U = 5.00$, $P < 0.0001$</i>	<i>$U = 0.00$, $P < 0.0001$</i>
Crops contents of bees at flowers	Volume		<i>$U = 600.00$, $P < 0.0001$</i>
	Concentration		<i>$U = 581.50$, $P < 0.0001$</i>
Swarming bees	Concentration	<i>$U = 0.00$, $P < 0.0001$ for all comparisons</i>	

We observed significant differences between the volume and concentration of residual nectar in the flowers and the nectar in the crops of bees captured at the flowers and returning to the hive at both Roodeplaat Nature Reserve (for volume $H_{2, 150} = 35.08$, $P < 0.001$; concentration $H_{2, 150} = 113.83$, $P < 0.001$) and Rust de Winter, including crop contents of swarming bees (volume $H_{2, 150} = 23.31$, $P < 0.001$; concentration $H_{3, 200} = 173.19$, $P < 0.001$). At Roodeplaat Nature Reserve the volume of residual nectar in individual flowers was significantly higher than the volume in the crops of bees captured at the flowers and in crops of foragers returning to the hive (Table 1, Fig. 1A). The crop volumes of bees captured at the flowers were not significantly higher than

those of foragers returning to the hive. There was a significant increase in concentration from the residual nectar to the crop contents of bees captured at the flowers, and a further increase in the crop of bees captured at the hive entrance (Table 1). The crop contents of returning foragers had a significantly higher concentration than those of bees captured at the flowers (Table 1).

The same pattern was observed at Rust de Winter (Fig. 1B). There was no significant difference between the volume of residual nectar in flowers and the crop volume of bees captured at the flowers, but the volume of residual nectar was significantly higher than the volume in the crops of returning foragers. The crop volumes of bees captured at the flowers were significantly higher than those of returning foragers (Table 1). The differences in concentration between the flowers and the crops of bees at the flowers, returning foragers and swarming bees remained highly significant after Bonferroni corrections. The concentration of residual nectar in the flowers was significantly lower than that in crop contents of bees captured at the flowers and of foragers returning to the hive (Fig. 1B). Nectar concentration in crops of returning foragers was significantly higher than that of bees captured at the flowers and the nectar concentration of swarming bees was significantly higher than all other measurements (Fig. 1B, Table 1).

Data at the two localities were collected on two consecutive days with similar weather. The temperature measured at Roodeplaat Nature Reserve increased from 22.2 to 28°C and RH decreased from 17.4 to 10.4%, and at Rust de Winter temperature increased from 18 to 25.4°C and RH from 27.6 to 15.1%.

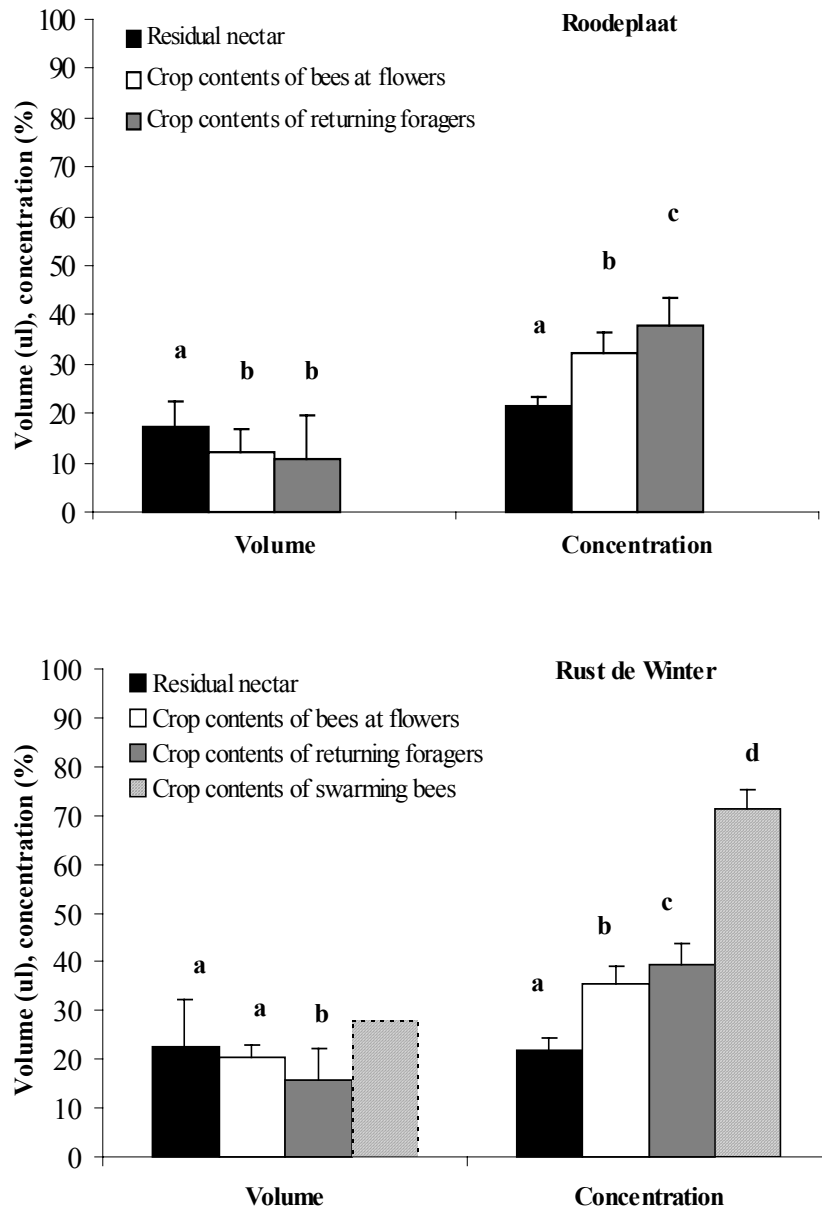


Figure 1. Nectar volume and concentration in residual nectar after honeybee visits and in crops of bees captured at the flowers and returning to the hive at (A) Roodeplaat and (B) Rust de Winter (means \pm SD, $n = 50$). Crop contents were collected and measured from bees captured at the flowers and at the hive entrance. Measurements at Rust de Winter included crop concentrations of swarming bees: the volume of crop contents for swarming bees was calculated (see text). No letters in common denote significant differences at $P < 0.025$ for Roodeplaat and $P < 0.017$ for Rust de Winter after Bonferroni corrections.

Discussion

Aloe greatheadii var *davyana* flowers when very few nectar and pollen sources are available. The flowers open throughout the day, ensuring constant availability of pollen to floral visitors. There is also constant availability of copious amounts of dilute nectar (Chapter 4). In spite of the nectar being more dilute than other bee-preferred nectars (30-50%, Southwick and Pimentel, 1981), this nectar flow contributes substantially to the honey crop (Williams, 2002) and serves as a source of water. According to Johansson and Johansson (1978), water availability may mean the difference between weak and strong colonies and access to water may result in increased brood rearing as observed in spring. The dilute nectar of *A. greatheadii* var *davyana* appears to meet the energy and water requirements of the bees during a dry period and may thus contribute to increased brood rearing.

Studies on crop loads carried by bees report both mass and volume. *Apis mellifera* *scutellata* bees in this study collected the most dilute nectar and carried the smallest loads compared to other reports (Table 2). According to Brosch and Schneider (1985), bees can store about 60 μ l in the crop with an unladen body mass of only 60 to 80 mg. The crop volumes measured in this study for bees at flowers (12 and 20 μ l) and those returning to the hive (11 and 16 μ l) are similar to the mean 13.6 μ l reported by Huang and Seeley (2003) for foragers when nectar was less abundant, but are much lower than those reported by Roubik & Buchmann (1984) for bees feeding on 45% sucrose solutions (59 μ l).

Foraging honeybees are able to regulate crop filling and it is known that the nectar load increases with a higher nectar flow rate (Núñez, 1970; Huang & Seeley, 2003) and with higher sugar concentrations (Roubik & Buchman, 1984). Seeley (1986) reported much larger crop loads (58 μ l) for bees returning from a feeder supplying a 55% sucrose solution than for bees returning from flowers (2 μ l). The observed partial crop loads in our study, in spite of readily available food sources and short flying distances to the hive, support the findings of Schmid-Hempel et al. (1985) who predicted partial crop loads for foragers flying short distances, thereby maximising their energetic efficiency rather than nectar delivery rate.

Table 2. Mass of bees and crop contents: departing foragers, crop contents after feeding on different diets and swarming bees.

Subspecies and locality	Diet	Body mass of honeybees (mg)	Mass of crop contents (mg)	Reference
<i>A. mellifera ligustica</i> Arizona	Feeder, 40%	73	23-29 max 70	Feuerbacher et al. (2003)
<i>A. mellifera ligustica</i> Japan	Natural sources Unknown	81	24 spring, summer 12 autumn	Fukuda et al (1969)
<i>A. mellifera scutellata</i> South Africa	<i>A. greatheadii</i> var <i>davyana</i> , 21%	62	12 - 14	This study
<i>A. mellifera scutellata</i> South Africa	<i>Eucalyptus</i> wide range	-	6 - 13	Hepburn & Magnuson (1988)
Africanised Various	Unknown	93	25 - 40	Winston (1987)
<i>A. mellifera</i> <i>lingustica</i> x <i>carnica</i> USA?	Natural sources 41%	-	40 - 80	Southwick and Pimentel (1981)
Swarming <i>A. mellifera</i> <i>scutellata</i> , South Africa	<i>A. greatheadii</i> var <i>davyana</i>	-	39	This study
Swarming Africanised Various	Unknown	-	30	Winston (1987)

An alternative reason for partial crop loads is a motivational one, where partial loads would instead serve to benefit the hive through increased exchange of information within the hive (recruitment of nestmates). The metabolic hypothesis as proposed by Schmid-Hempel et al. (1985) lost support when Moffat (2000) found that metabolic rates of bees were unrelated to the size of crop loads but were linearly related to the reward rate and might be controlled by a motivational drive. In contrast, Wolf et al. (1989) and Feuerbacher et al. (2003) reported increased metabolic rates for honeybees with an increased nectar load. Territorial male bees avoid carrying large crop loads, as illustrated by the small loads carried by male carpenter bees (20 μ l) compared to females (69 μ l) (Louw & Nicolson, 1983) and male *Anthophora plumipes* that only carry enough nectar (1-5 μ l) to meet their short term requirements, while females commonly carry 30 μ l nectar for their own needs and that of their offspring (Willmer & Stone, 2004).

The increase in concentration of the crop contents that we observed is already apparent in honeybees collected at the flowers. This increase in nectar concentration is contrary to the findings of Park (1932), who found that nectar is not concentrated in the crop of bees returning to the hive, but only in the hive itself during the storage and honey ripening process. It is known that departing foragers do not leave the hive with completely empty crops (Park, 1932; Lindauer, 1955; Winston, 1987) and the amounts in crops of departing bees may vary from bee to bee and with time (Park, 1932). However, both Park (1932) and Oertel et al. (1951) starved their bees for at least an hour before conducting their experiments. This meant that bees had no nectar or honey in their crops prior to feeding on the sugar solutions offered. According to Oertel et al. (1951), a decrease in concentration in the crop is to be expected since bees add dilute glandular secretions, including the enzyme invertase, to the crop contents, resulting in both dilution and hydrolysis of sucrose into glucose and fructose. However, *A. greatheadii* var *davyana* produces 98% hexose nectar (Van Wyk et al., 1993), and no hydrolysis is necessary.

Our findings regarding changes in crop concentration are in agreement with those of Willmer (1986, 1988), who also investigated changes in nectar concentration after collection. Willmer (1986) investigated mason bees, *Chalicodoma sicula*, collecting dilute *Lotus creticus* nectar (daily range 22-40%) growing on sand dunes in Israel. The bees rapidly increased their crop contents to about 58% after collection of nectar. Since a simultaneous dilution of the haemolymph was measured, water must have been transported from the gut into the haemolymph. Willmer (1988) also studied two species of carpenter bees, *Xylocopa sulcatipes* and *X. pubescens*, collecting nectar from *Calotropis procera* in southern Israel. Crop contents of the smaller *X. sulcatipes* bees (57%) were much more concentrated than nectar in the flowers or the crop contents of *X. pubescens* (46%). In *X. sulcatipes* bees, water moved from nectar in the gut into the haemolymph during flight, thus is lowering their haemolymph concentrations. The only other study that has examined changes in nectar concentrations in bee crops is that of Biesmeijer et al. (1999), who compared the crop contents of two *Melipona* species collecting 50% sucrose from an artificial feeder in Costa Rica. He observed that sugar concentration of the load increased by only 0.2% between the feeder and the hive.

The crop or honey stomach of bees is an expandable compartment that stores honey as well as nectar and water collected by foragers. Its primary function is to retain nectar or water and in addition it is the site where invertase is added to nectar to hydrolyse sucrose (Lindauer, 1955; Louw & Nicolson, 1983). However, foragers need enough energy to sustain flight. It is energetically advantageous for them to use fuels stored in the crop rather than reserves stored in fat body or muscle. It is assumed that bees can only use nectar stored in the crop when it passes through to the midgut, since the crop is impermeable (its cuticular lining prevents absorption of either sugar or water molecules; Lindauer, 1955). Crop emptying in bees is controlled through the osmolality of the food and haemolymph and adjusted to energy demands (Roces & Blatt, 1999). Foragers are able to adjust the rate at which sugar leaves the crop according to their metabolic rates (Blatt & Roces, 2002) and it is known that the metabolic rates in turn depend on the reward rate at the food source (Balderrama et al., 1992).

Excess water can be withdrawn from nectar either internally via the midgut or externally through evaporation from the mouthparts. "Tongue-lashing" is a process where nectar is regurgitated onto the tongue and evaporated, and is used by honeybees to achieve evaporative cooling of the body, in particular the head (Heinrich, 1980), a process effectively used by *A. mellifera caucasica* bees flying in the Sonoran desert (Cooper et al., 1985) or in *Xylocopa* bees to concentrate nectar before storage (Corbet & Willmer, 1980). The excretion of copious urine, whether in flight or when alighting on a flower, is conspicuous in carpenter bees, *Xylocopa* species (Willmer, 1988; Nicolson, 1990) and bumble bees, *Bombus lucorum* (Bertsch, 1984) and, according to Park (1932), it is well known that honeybees also excrete a colourless liquid, believed to be water, when transporting dilute nectar. Johansson and Johansson (1978) reported that water-collecting bees regurgitate only 70% of the water collected, while the remaining 30% is ingested and removed through excretion. The removal of water will explain the increase in concentration and the decrease in volume of the crop contents. The small volumes transported back to the hive in our study will aid the concentrating process since removal of water will have more of an effect on small volumes. However, since the crop is impermeable it is difficult to explain the removal of water from the crop contents without accompanying sugar.

Honeybees foraging on the dilute nectar of *A. greatheadii* var *davyana* are flying in very dry air, therefore evaporative losses during flight may be considerable. The bees may thus forage partly to get enough water for their physiological needs. The low concentration of *A. greatheadii* var *davyana* nectar is not a problem for water balance at the colonial level because evaporation of the dilute nectar is aided by low ambient humidities prevailing during the flowering season.

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References

- Balderrama, N.M., Almeida de B., L.O. & Núñez, J.A. (1992) Metabolic rate during foraging in the honeybee. *Journal of Comparative Physiology* **162**: 440-447.
- Biesmeijer, J.C., Richter, J.A.P., Smeets, M.A.J.P. & Sommeijer, M.J. (1999) Niche differentiation in nectar-collecting stingless bees: the influence of morphology, floral choice and interference competition. *Ecological Entomology* **24**: 380-388.
- Bertsch, A. (1984) Foraging in male bumblebees (*Bombus lucorum* L.): maximizing energy or minimizing water load? *Oecologia* **62**: 325-336.
- Blatt, J. & Roces, F. (2002) The control of the proventriculus in the honeybee (*Apis mellifera carnica* L.) II Feedback mechanisms. *Journal of Insect Physiology* **48**: 683-691.
- Brosch, U. & Schneider, L. (1985) Fine structure and innervation of the honey stomach (crop) of the honeybee, *Apis mellifera* L. (Hymenoptera: Apidae). *International Journal of Insect Morphology and Embryology* **14**: 335-345.
- Cooper, P.D., Schaffer, W.M. & Buchman, S.L. (1985) Temperature regulation of honeybees (*Apis mellifera*) foraging in the Sonoran desert. *Journal of Experimental Biology* **114**:1-15.
- Corbet, S.A. & Willmer, P.G. (1980) Pollination of the yellow passion fruit: nectar, pollen and carpenter bees. *Journal of Agricultural Science* **95**: 655-666.
- Eisikowitch, D. & Masad, Y. (1982) Preferences of honeybees for different ornamental nectar-yielding plants during the dearth period in Israel. *Bee World* **63**: 77-82.
- Feuerbacher, E., Fewell, J.H., Roberts, S.P., Smith, E.F. & Harrison, J.F. (2003) Effects of load type (pollen or nectar) and load mass on hovering metabolic rate and mechanical power output in the honeybee *Apis mellifera*. *Journal of Experimental Biology* **206**: 1855-1865.
- Fukuda, H., Moriya, K. & Sekiguchi, K. (1969) The weight of crop contents in foraging honeybee workers. *Annotationes Zoologicae Japonenses* **42**: 80-90.
- Glen, H.F. & Hardy, D.S. (2000) Fascicle 1: Aloaceae (First Part): *Aloe* In Germishuizen, G. (Ed) *Flora of Southern Africa* **5** National Botanical Institute, Pretoria.
- Heinrich, B. (1980a). Mechanisms of body-temperature regulation in honeybees, *Apis mellifera*. I. Regulation of head temperatures. *Journal of Experimental Biology* **85**: 61-72.

- Hepburn, H.R. & Magnuson, P.C. (1988) Nectar storage in relation to wax secretion by honeybees. *Journal of Apicultural Research* **27**: 90-94
- Hoffman, M.T. (1988) The pollination ecology of *Aloe ferox* Mill. *South African Journal of Botany* **54**: 345-350.
- Huang, M.H. & Seeley, T.D. (2003) Multiple unloadings by nectar foragers in honey bees: a matter of information improvement or crop fullness? *Insectes Sociaux* **50**: 1-10.
- Jackson, M.E. (1979) A visit to the aloes. *South African Bee Journal* **51**: 22-23.
- Johansson, T.S.K. & Johansson, M.P. (1978) Providing honeybees with water. *Bee World* **59**: 11-17.
- Lindauer, M. (1955) The water economy and temperature regulation of the honeybee colony. *Bee World* **36**: 62-111.
- Louw, G.N. & Nicolson, S.W. (1983) Thermal, energetic and nutritional considerations in the foraging and reproduction of the carpenter bee *Xylocopa capitata* *Journal of the Entomological Society of Southern Africa* **46**: 227-240.
- Moffat, L. (2000) Changes in the metabolic rate of the foraging honeybee: effect of the carried weight or the reward rate? *Journal of Comparative Physiology* **186**: 299-306.
- Nicolson, S.W. (1990) Osmoregulation in a nectar feeding insect, the carpenter bee *Xylocopa capitata*: water excess and ion conservation. *Physiological Entomology* **15**: 433-440.
- Nicolson, S.W. (2002) Pollination by passerine birds: why are the nectars so dilute? *Comparative Biochemistry Physiology B* **131**: 645-652.
- Nicolson, S.W. & Nepi, M. (2005) Dilute nectar in dry atmospheres: nectar secretion patterns in *Aloe castanea* (Asphodelaceae). *International Journal of Plant Science* **166**: 227-233.
- Núñez, J.A. (1970) The relationship between sugar flower and foraging and recruiting behaviour of honeybees (*Apis mellifera* L.). *Animal Behaviour* **18**: 527-538.
- Oertel, E., Fieger, E.A., Williams, V.R. & Andrews, E.A. (1951) Inversion of cane sugar in the honey stomach of the bee. *Journal of Economic Entomology* **44**: 487-492.
- Ohguchi, O. & Aoki, K. (1983) Effects of colony need for water on optimal food choice in honeybees. *Behavioral Ecology and Sociobiology* **12**: 77-84.

- Park, O.W. (1932) Studies on the changes in nectar concentration produced by the honeybee, *Apis mellifera*. Part I. Changes which occur between the flower and the hive. *Research Bulletin of the Iowa Agricultural Experiment Station* **151**: 211-243.
- Roces, F. & Blatt, J. (1999) Haemolymph sugars and the control of the proventriculus in the honey bee *Apis mellifera*. *Journal of Insect Physiology* **45**: 221-229.
- Roubik, D.W. & Buchmann, S.L. (1984) Nectar selection by *Melipona* and *Apis mellifera* (Hymenoptera: Apidae) and the ecology of nectar intake by bee colonies in a tropical forest. *Oecologia* **61**: 1-10.
- Roubik, D.W., Yanega, D., Aluja, M., Buchmann, S.L. & Inouye, DW (1995) On optimal nectar foraging by some tropical bees (Hymenoptera, Apidae). *Apidologie* **26**:197-211.
- Schmid-Hempel, P., Kacelnik, A. & Houston, A.I. (1985) Honeybees maximize efficiency by not filling their crop. *Behavioral Ecology and Sociobiology* **17**: 61-66.
- Seeley, T.D. (1986) Social foraging by honeybees: how colonies allocate foragers among patches of flowers. *Behavioral Ecology and Sociobiology* **19**: 343-354.
- Southwick, E.E. & Pimentel, D. (1981) Energy efficiency of honey production by bees. *BioScience* **31**: 730-732.
- Statistica (1984-2004) *StatSoft Inc.* Tulsa, USA.
- Van Wyk, B-E., Whitehead, C.S., Glen, H.F., Hardy, D.S., van Jaarsveld, E.J. & Smith, G.F. (1993) Nectar sugar composition in the Subfamily Alooideae (Asphodelaceae). *Biochemical Systematics and Ecology* **21**: 249-253.
- Van Wyk, B-E. & Smith, G. (1996) *Guide to Aloes of South Africa*. Briza Publications, Pretoria.
- Visscher, P.K. & Seeley, T.D. (1982) Foraging strategy of honeybee colonies in a temperate deciduous forest. *Ecology* **63**: 1790-1801.
- Williams, J. (2002) The aloe flowering season: the most interesting honey-flow for South African beekeepers. *South African Bee Journal* **74**: 3-9.
- Willmer, P.G. (1986) Foraging patterns and water balance: problems of optimization for a xerophilic bee, *Chalicodoma sicula*. *Journal of Animal Ecology* **55**: 941-962.
- Willmer, P.G. (1988) The role of insect water balance in pollination ecology: *Xylocopa* and *Calotropis*. *Oecologia* **76**: 430-438.

- Willmer, P.G. & Stone, G.N. (2004) Behavioural, ecological, and physiological determinants of the activity patterns of bees. *Advances in the Study of Behavior* **34**: 347-466.
- Winston, M.L. (1987) *The biology of the honey bee* Harvard University Press, London.
- Wolf, T.J., Schmid-Hempel, P., Ellington, C.P. & Stevenson, R.D. (1989) Physiological correlates of foraging efforts in honeybees: Oxygen consumption and nectar load. *Functional Ecology* **3**: 417-424
- Zar, J.H. (1984) *Biostatistical analysis*. Prentice-Hall, New Jersey.

CHAPTER 6

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 impairs 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). This water is collected either by water foragers or incidentally through foraging for nectar (Lindauer, 1955; Kühnholz & Seeley, 1997).

In spite of the supposedly important role of humidity in brood development (Park, 1949; Lindauer, 1955), little is known of how this parameter is regulated by honeybees, if at all (Ribbands, 1953; Büdel, 1960; Simpson, 1961; Johansson & 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, in order to accommodate large monitoring devices such as hygrothermographs (e.g. Oertel, 1949). Usually the measurements were of relative humidity, which is dependant 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.

Methods

We measured temperature, absolute humidity (AH) and relative humidity (RH) inside three *Apis mellifera scutellata* colonies containing approximately 20,000 bees each reared in Langstroth hives with one shallow super. AH was measured in order 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 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 one kilometre of a dam, providing them with a source of water.

Miniature HOBO H8 data loggers (61 x 48 x 20 mm, Onset Computer Corporation, Pocasset, MA, USA) were used for continuous recording of temperature, AH and RH (at 2-min intervals for four consecutive days). The operating ranges of the loggers for RH, AH and temperature are 25 to 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°C, 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 four 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 two days monitored. Bonferroni correction was applied when the parameters measured were compared for paired combinations of the three colonies. 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 (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 ($df = 3, n = 378, F > 19.6, P < 0.001$ in all cases). There were also significant differences in AH between the brood areas of different colonies as well as between their nectar stores ($df = 3, n = 378, F > 17.8, P < 0.01$ after Bonferroni correction in all cases). The same was true for RH ($df = 3, n = 378, F > 23.3, P < 0.01$ after Bonferroni correction in all cases).

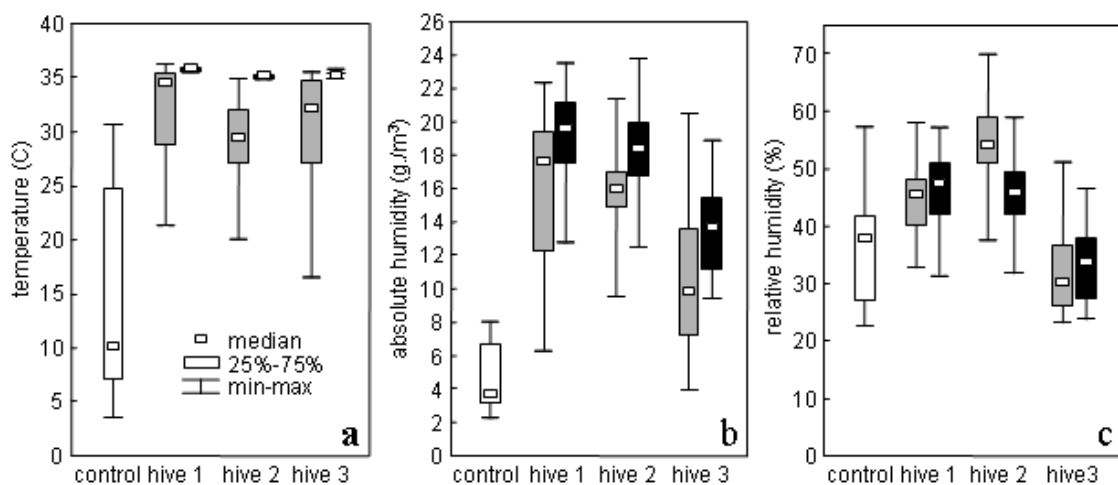


Figure 1. Summary statistics for microclimatic parameters in three colonies measured over two consecutive days with similar weather. Data shown are (a) temperature, (b) absolute humidity and (c) relative humidity in the nectar stores (grey bars) and in the brood area (black bars). Parameters measured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

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 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).

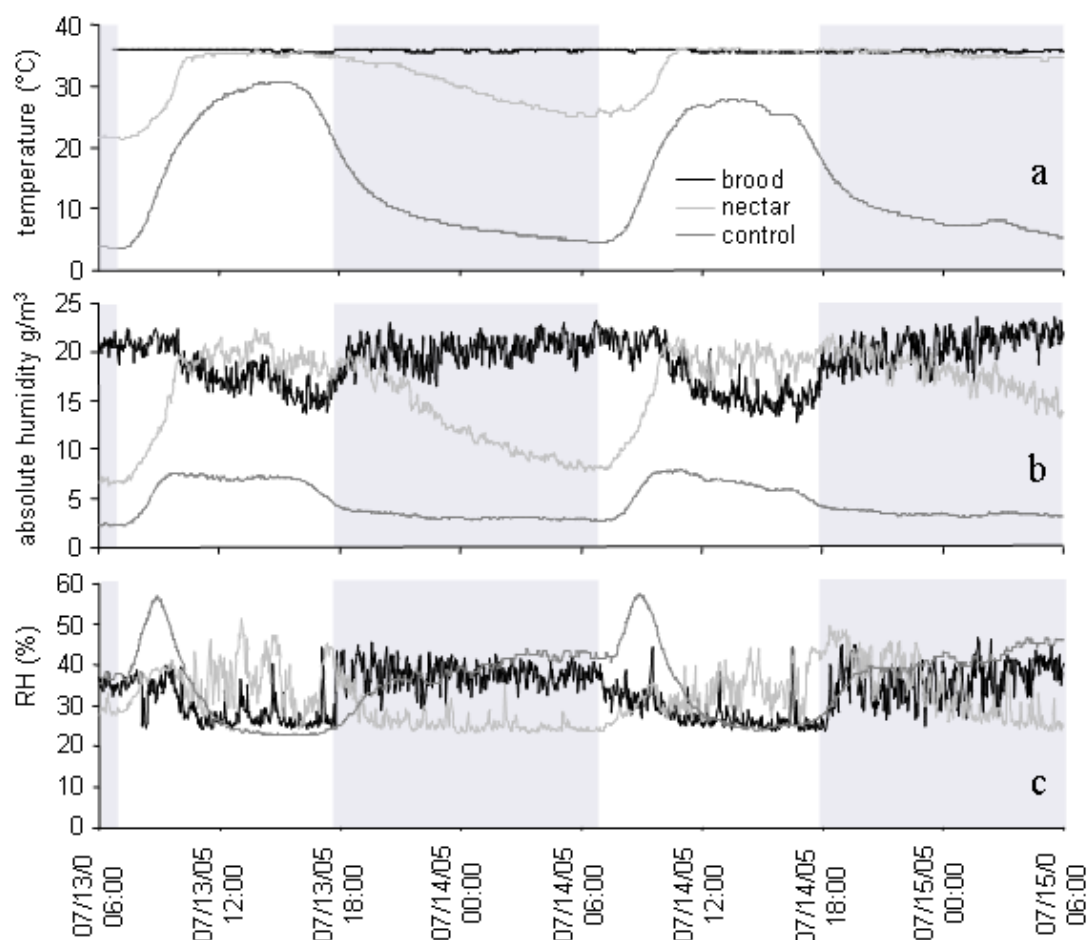


Figure 2. Variation in microclimatic parameters in a single colony over two consecutive days (only data for two days are presented for clarity). Data shown are (a) temperature, (b) absolute humidity and (c) relative humidity in the nectar stores and in brood area. Results obtained were similar for all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

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 (see literature in Moritz & 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 (*Aloe greatheadii* var *davyana* nectar has a water content of 77%; (Chapter 4). Decreasing humidity in these stores would allow the evaporation of nectar in 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 & 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 (see above). 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 & Linsenmair, 1998; Kleineidam & 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 & 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 & 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 (approx. 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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References

- Büdel, A. (1960) Bienenphysik. In: Büdel, A., Herold, E. (Eds.), *Biene und Bienezucht* pp 115-180, Ehrenwirth Verlag, München.
- Bujok, B., Kleinhenz, M., Fuchs, S. & Tautz, J. (2002) Hot spots in the bee hive. *Naturwissenschaften* **89**: 299-301.
- Degrandi-Hoffman, G., Spivak, M. & Martin, J.H. (1993) Role of thermoregulation by nestmates on the development time of honey bee (Hymenoptera: Apidae) queens. *Annals of the Entomological Society of America* **86**: 165-172.
- Doull, K.M. (1976) The effects of different humidities on the hatching of the eggs of honeybees. *Apidologie* **7**: 61-66.
- Heinrich, B. (1993) *The hot-blooded insects: strategies and mechanisms of thermoregulation*. Harvard University Press, Cambridge.
- Himmer, A. (1927) Ein Beitrag zur Kenntnis des Wärmehaushalts im Nestbau Sozialer Hautflüger. *Zeitschrift für Vergleichende Physiologie* **5**: 375-389.
- Johansson, T.S.K. & Johansson, M.P. (1979) The honeybee colony in winter. *Bee World* **60**: 155-169.
- Kleineidam, C. & Roces, F. (2000) Carbon dioxide concentrations and nest ventilation in nests of the leaf-cutting ant *Atta vollenweideri*. *Insectes Sociaux* **47**: 241-248.
- Korb, J. & Linsenmair, K.E. (1998) The architecture of termite mounds: a result of a trade-off between thermoregulation and gas exchange? *Behavioural Ecology* **10**: 312-316.
- Kühnholz, S. & Seeley, T.D. (1997) The control of water collection in honey bee colonies. *Behavioural Ecology and Sociobiology* **41**: 407-422.
- Lindauer, M. (1953) Division of labour in the honeybee colony. *Bee World* **34**: 63-73, 85-90.
- Lindauer, M. (1955) The water economy and temperature regulation of the honeybee colony. *Bee World* **36**: 62-111.
- Moritz, R.F.A. & Southwick, E.E. (1992) *Bees as superorganisms: an evolutionary reality*. Springer-Verlag, Berlin.
- Nelson, W., Tong, Y.L., Lee, J.K. & Halberg, F. (1979) Methods for cosinor rhythmicity. *Chronobiologia* **6**: 305-323.
- Oertel, E. (1949) Relative humidity and temperature within the beehive. *Journal of Economic Entomology* **42**: 528-531.

- Park, O.W. (1949) In: Dadant and sons (Eds.), *The hive and the honeybee*. Hamilton, Illinois.
- Ribbands, C.R. (1953) *The behaviour and social life of honeybees*. Bee Research Association, London, UK.
- Seeley, T.D. (1974) Atmospheric carbon dioxide regulation in honeybee (*Apis mellifera*) colonies. *Journal of Insect Physiology* **20**: 2301-2305.
- Simpson, J. (1961) Nest climate regulation in honey bee colonies. *Science* **133**: 1327-1333.
- Southwick, E.E. & Moritz, R.F.A. (1987) Social control of air ventilation in colonies of honey bees, *Apis mellifera*. *Journal of Insect Physiology* **33**: 623-626.
- Stabentheiner, A., Pressl, H., Papst, T., Hrassnigg, N. & Crailsheim, K. (2003) Endothermic heat production in honeybee winter cluster. *Journal of Experimental Biology* **206**: 353-358.
- Starks, P.T. & Gilley, D.C. (1999) Heat shielding: a novel method of colonial thermoregulation in honey bees. *Naturwissenschaften* **86**: 438-440.
- Willmer, P.G. (1986) Microclimate and the environmental physiology of insects. *Advanced Insect Physiology* **16**: 1-57.
- Wohlgemuth, R. (1957) Die Temperaturregulation des Bienenvolkes unter regeltheoretischen Gesichtspunkten. *Zeitschrift für Vergleichende Physiologie* **40**: 119-161.