

Review

Water homeostasis in bees, with the emphasis on sociality

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Summary

Avenues of water gain and loss in bees are examined here at two levels of organisation: the individual and the colony. Compared with the majority of terrestrial insects, bees have a high water turnover. This is due to their nectar diet and, in larger species, substantial metabolic water production during flight, counteracted by high evaporative and excretory losses. Water fluxes at the colony level can also be very high. When incoming nectar is dilute, honeybees need to remove large volumes of water by evaporation. On the other hand, water is not stored in the nest and must be collected for evaporative cooling and for feeding the brood. Water regulation has many similarities at individual and colony levels. In particular, manipulation of nectar or water on the tongue is extensively used by bees to increase evaporation for either food-concentrating or cooling purposes.

Key words: nectar, bubbling, water collection, evaporation.

Why bees?

Bees are not normally model insects for studying water balance. Bee physiology is characterised by endothermy in even small species (Stone and Willmer, 1989) and is complicated by the social context. Bees show the full spectrum of sociality: although most are solitary it is the social bumblebees and honeybees whose physiology and behaviour are most familiar to us (Willmer and Stone, 2004). In addition, the sophisticated thermal physiology of bees has received much more attention (e.g. Heinrich, 1985; Kovac et al., 2007) than their water regulation. Here I briefly examine the water balance physiology of bees, considering the usual avenues of water gain and loss, at two levels of organisation: the individual and the colony, sometimes termed a ‘superorganism’ (Moritz and Southwick, 1992). It is accepted that social homeostasis can be explained by the coordinated activity of bees as individuals, responding to their internal or external environment, without the need to invoke centralised control (Jones and Oldroyd, 2007; Seeley, 1995).

Water fluxes of honeybees can be very high at the colony level. For example, Seeley estimated average annual requirements of 120 kg of nectar, 20 kg of pollen and 25 litres of water for a single wild colony (Seeley, 1995). This applies to honeybees in cold temperate conditions, and the estimate for nectar includes substantial energy stores required for over-wintering. The seasonal cycles of African honeybees are limited by rainfall, not temperature, and better foraging weather means that in comparison massive honey stores are not needed (Hepburn and Radloff, 1998).

Water gain in food

Individual

While male bees of most species take care of their own energetic needs, all female bees forage far beyond their individual needs, collecting pollen and nectar as provisions for the offspring they raise; therefore activity patterns of the sexes may be very different (Willmer and Stone, 2004). Where comparisons have been made, male *Xylocopa capitata* and *Anthophora plumipes* carry much

smaller crop loads than do females (Louw and Nicolson, 1983; Stone, 1995). The impermeable crop expands greatly for storage and transport of nectar and protects the haemolymph from osmotic shock. *Apis mellifera* workers can carry close to their body mass in nectar, although crop loads tend to be much smaller (Schmid-Hempel et al., 1985) and are positively correlated with the nectar concentration (Hunt et al., 1995). This suggests that under natural conditions honeybees might carry less than during experiments in which they are fed concentrated sugar syrup (e.g. Park, 1932; Waller, 1972). Their feeding system is designed to retain nectar in the crop for as long as possible. Sugar leaves the crop at a rate dependent on the metabolic rate of the individual, but the fluid-emptying rate (and the rate of rectal filling) are inversely proportional to nectar concentration (Roces and Blatt, 1999).

Two factors – the body size of bees and environmental conditions – strongly influence their need to obtain water from nectar. Large bees require concentrated nectar at low to moderate ambient temperature (T_a) because of their very high metabolic water production in flight (Bertsch, 1984; Nicolson and Louw, 1982). In contrast, smaller desert bees in Israel (mason bees and carpenter bees) use dilute nectar for rehydration purposes, as shown by field measurements of decreasing haemolymph osmolality after ingestion of nectar (Willmer, 1986; Willmer, 1988). For even smaller bees in less arid environments, dilute nectar imposes a high water load. After drinking the dilute (14% w/w) nectar of *Aloe arborescens*, female allodapine bees, *Allodapula variegata* and *Braunsapis* sp., concentrate it on the tongue by repeated regurgitation, evaporation and re-ingestion (Fig. 1) (M. B. Ellis and S.W.N., unpublished), presumably before mixing it with pollen and feeding it to their larvae (allodapine bees are characterised by progressive provisioning of the brood). This is analogous to ‘bubbling’ or oral droplet extrusion behaviour in fruit-feeding tephritid flies (Hendrichs et al., 1992). The same behaviour has been seen in *Hylaeus heraldicus* (Colletidae) after collecting dilute nectar of *Kniphofia* sp. (Nicolson, 1998). Other reports of nectar dehydration are in solitary and social halictine bees (Michener,



Fig. 1. Female *Allodapula variegata* concentrating the dilute (14%) nectar of *Aloe arborescens* by evaporation. The regurgitated droplet, held under the tongue, is repeatedly sucked in and out and may be very large in relation to the size of the bee (body length 7 mm). Photo, Michael Ellis.

1974). The behaviour is not confined to females: laboratory-fed male bumblebees evaporate nectar on their tongues when given 30% instead of 50% sucrose (Bertsch, 1984), and male carpenter bees *Xylocopa nigrocincta* are fed nectar by females, then dehydrate it at the nest entrance, thus improving the efficiency of territorial flight (Wittmann and Scholz, 1989). Stingless bee males form large congregations outside the nest where they dehydrate nectar on their tongues (Cortopassi-Laurino, 2007). In all these examples, oral elimination of excess water compensates for less than ideal nectar concentrations.

Fresh pollen is relatively dehydrated after exposure at anthesis, but its water content increases after collection by bees due to the addition of nectar and glandular secretions (Human and Nicolson, 2006). The larval diets of bees vary greatly in water content (Roubik, 1989). The water content of royal jelly is around 67% (Wongchai and Ratanavalachai, 2002), but the larval food of stingless bees contains less water, a thicker consistency being necessary for larvae floating on top of mass provisions (Hartfelder and Engels, 1989). Large carpenter bees provision their nests with semi-solid masses of pollen combined with nectar, giving a final water content of only 20% in the provisions of *X. capitata* (Louw and Nicolson, 1983). In *Xylocopa mordax*, nectar is pre-concentrated on the tongue for this purpose (Corbet and Willmer, 1980).

Colony

Honeybees prefer sugar concentrations of 30–50% (sugar concentrations here are given as % w/w as in refractometer measurements) under experimental conditions (Waller, 1972), but in practice they collect from a much wider range of nectars. Seeley measured 15–65% in nectar loads being brought into a single colony (Seeley, 1986), and Hunt and colleagues recorded a similar range of concentrations in incoming loads (Hunt et al., 1995). The choice of nectar concentration depends on the ecological context, i.e. on the other food sources available at the time. This has long been a complicating factor in experimental studies of honeybee foraging behaviour. For example, Lindauer found that the threshold sucrose concentration for eliciting recruitment behaviour declined from 55% to 4% as the German summer progressed (Lindauer, 1948). Empirical measurements of energy intake rate in bees show

peak values at sucrose concentrations around 60% in bumblebees, stingless bees and honeybees (Harder, 1986; Roubik and Buchmann, 1984). Note that for orchid bees (Euglossini), which use suction feeding rather than a lapping mechanism, optimal concentrations are lower and more dilute nectars are collected (Borrell, 2004).

Communal food storage requires that the osmolality of honey is high enough to inhibit microbial growth (Pusey, 1999). In honeybees this is achieved first by hydrolysis of nectar sucrose to glucose and fructose, through the addition of hypopharyngeal gland enzymes, and then through evaporative processing by food-handling bees to reach a concentration of about 82%. These bees evaporate nectar on their tongues before placing droplets in open cells for further evaporation, accelerated by fanning (Park, 1925). Among stingless bees, workers drink water condensed in the nest during honey ripening and regurgitate it outside the entrance (Roubik, 2006). Their ripened honey is around 70% in concentration and tends to ferment (Cortopassi-Laurino et al., 2006; Roubik, 2006). Note that uncapped honey is hygroscopic and absorbs water, so can be both a sink and source of water in the nest.

Trophallaxis is the regurgitation of the crop contents of a donor bee for ingestion by receiver bees. Extremely rapid distribution of incoming nectar was demonstrated by Nixon and Ribbands, who fed radiolabelled food to six foragers and were able to detect the label in 62% of all foragers after only 4 h and in all large larvae in unsealed cells after 48 h (Nixon and Ribbands, 1952). Trophallactic interactions ensure that homeostasis is achieved in the 'collective stomach' of all workers, which is a nectar reserve for the colony (Schmickl and Crailsheim, 2004). Similarly, colonies preparing to swarm store concentrated food in their crops, comprising 20–30% of the mass of individuals and of the swarm (Combs, 1972). In addition to its nutritional significance, liquid transfer between adults is a means of exchange of information about the quality and quantity of food reserves in the colony (Crailsheim, 1998).

Drinking/water collecting

Individual

It is not clear whether solitary bees drink water for their own needs, as distinct from seeking dilute nectar. Large aggregations of bees of various genera can be seen foraging at wet soil substrates in the tropics, then regurgitating and reimbibing fluid, and this may be a means of obtaining salts (Roubik, 1989). Although bumblebees are not expected to drink, marked *Bombus terrestris* were observed drinking repeatedly from a water trough during warm conditions (Ferry and Corbet, 1996): it is unlikely that individual bumblebees would have a water deficit so this was probably for the benefit of the colony. When groups of 100 honeybees are confined in cages and provided with 67% sugar, they drink about 10 μ l of water daily at T_a of 35°C and 40°C (Free and Spencer-Booth, 1958).

Colony

Honeybee colonies collect water for two reasons, related to different types of weather: for cooling of the brood area by evaporation on hot days, and for feeding the larval brood when foraging is limited on cool days (Lindauer, 1955; Seeley, 1995). The classic studies of Lindauer showed how bees regulate the hive temperature in hot conditions (Lindauer, 1955). Water is collected by water foragers, then distributed around the hive and in cells containing eggs and larvae; fanning accelerates its evaporation, as does regurgitation and evaporation on the tongue (Lindauer, 1955). Visscher and colleagues measured mean water loads of 44 mg in honeybees collecting water under desert conditions (Visscher et al.,

1996). Paper wasps and hornets also use water for cooling their nests, but the highly social stingless bees do not (Jones and Oldroyd, 2007; Roubik, 2006).

The second need for water – for consumption by nurse bees when feeding the brood – is an aspect of water use by honeybees that tends to be underestimated (Johansson and Johansson, 1978). Nurse bees feed young larvae a secretion from their hypopharyngeal glands; for worker larvae after the third day this jelly is supplemented with honey and pollen (Crailsheim, 1998). As already mentioned, the water content of royal jelly is high, so nurse bees have a great need for water when brood rearing is intensive; this water cannot always be obtained from nectar.

The regulation of water collection in honeybees is discussed in detail by Seeley (Seeley, 1995). In essence, the rate of unloading of water foragers indicates the colony demand for water (i.e. the feedback system is similar to that for nectar). In this way the balance between collection and consumption of water is maintained. Importantly, water collection does not interfere with the collection of concentrated nectar by the colony (Kuhnholz and Seeley, 1997). The first bees to start water collection may be stimulated by the collective increase in crop sugar concentration of all bees in the nest, due to trophallaxis (Lindauer, 1955; Seeley, 1995), or possibly by the collective increase in haemolymph osmolality. Apart from environmental factors, the tendency of honeybee foragers to collect water, nectar or pollen has a genetic component (Hunt et al., 1995). Workers with the lowest sucrose response thresholds, i.e. those able to distinguish low sucrose concentrations from water in proboscis extension response tests, become water foragers (Pankiw and Page, 2000).

Water foraging is regulated according to current demand and water is not stored in combs by temperate honeybee colonies: this is because nectar availability fluctuates widely and water sources usually do not (Seeley, 1995). For African honeybees, occasional water storage has been recorded in wild bee nests, as after summer rain in the Kalahari Desert (Eksteen and Johannsmeier, 1991). Park recorded temporary storage of water in the crops of ‘reservoir bees’ (Park, 1923).

Metabolic water gains

Individual

Studies on the mechanisms by which bees thermoregulate in flight have yielded data on the relative magnitudes of metabolic water gains and evaporative water losses for bees as individuals. Metabolic water production by large flying bees (*Xylocopa* and *Bombus*) is substantial (Bertsch, 1984; Nicolson and Louw, 1982), especially at low or moderate T_a . Honeybees ferrying large water loads to the hive at high T_a produce enough metabolic water to offset their evaporative water losses (Louw and Hadley, 1985). However, it cannot be assumed that metabolic gains will balance evaporative losses in flight. Because variation in metabolic heat production is used by bees (*Apis* and *Centris*) as a primary mechanism of thermoregulation in flight (Roberts and Harrison, 1999; Roberts et al., 1998), at high T_a metabolic water production will decrease as evaporative water loss increases. For individual bees, the thermal environment is crucial to water balance in flight.

Colony

Bees engaged in brood warming generate metabolic heat using their flight muscles. At low T_a , the metabolic rates of incubating bumblebees are extremely high (Heinrich, 1974). Honeybee brood nest temperatures are maintained constant at about 35°C by bees that ‘shiver’ on the comb surface or inside empty cells in the brood

area (Kleinhenz et al., 2003), and high metabolic water production can be assumed during this energy-intensive heating activity. However, the general colony heat production to which all workers contribute does not require much increase in metabolism (Harrison, 1987).

Evaporative losses

Individual

In flying *A. mellifera* and *Centris pallida* there is a negative relationship between water balance and T_a , the bees being in negative water balance at T_a above 31°C (Roberts and Harrison, 1999; Roberts et al., 1998). Although the cuticular permeability of both species increases with T_a , neither cuticular nor respiratory water losses are sufficient to explain the very high evaporative water losses at high T_a . These probably involve cooling mechanisms such as regurgitation of crop contents onto the proboscis (Heinrich, 1980). This ‘tongue lashing’ at high T_a has been observed in a variety of bees (Roberts and Harrison, 1998) and in honeybees has been shown to increase evaporative losses dramatically (Louw and Hadley, 1985). As a result, the thoracic temperatures of nectar and water foragers are significantly lower than those of pollen foragers (Cooper et al., 1985; Feuerbacher et al., 2003).

Colony

The amount of water that has to be evaporated from dilute nectar is enormous (Fig. 2). In order to increase the sugar concentration from 20% to 82%, bees must evaporate 0.75 g water for every 1 g of nectar collected, and the mass of honey produced from a given mass of nectar is correspondingly reduced. Recently we have shown that foragers of *A. mellifera scutellata* collecting dilute nectar of *Aloe greatheadii* var. *davyana* in dry winter air begin to concentrate the nectar before returning to the hive (Nicolson and Human, 2008). Because the crop is impermeable to both sugar and water, we can only explain the doubling of crop sugar concentration, from 20% to 40%, by evaporation on the tongue. This contradicts the conventional wisdom that the concentration of nectar is unchanged during its transport by bees between flowers and the hive (Park, 1932). The advantage for the bees lies in reducing the water load that has to be carried and the amount of

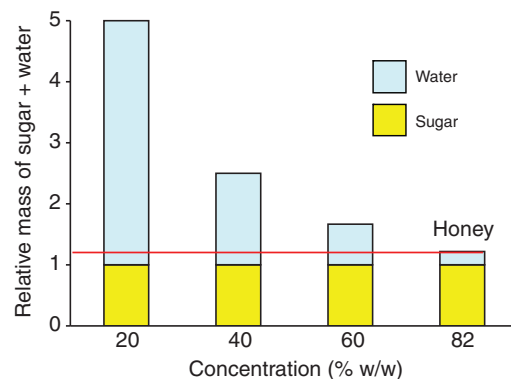


Fig. 2. Relative masses of sugar and water in nectars of various concentrations and in honey (horizontal red line). The figure shows the nectar-processing advantage for *Apis mellifera scutellata* workers concentrating the nectar of *Aloe greatheadii* var. *davyana* from 20% to 40% on their tongues: two-thirds of the necessary evaporation is achieved before the bees return to the hive (Nicolson and Human, 2008).

evaporation needed in the hive (Fig. 2); the cooling effect is less desirable for individual foragers but disturbances of heat or water balance can be corrected in the hive.

The various processes involved in cooling the honeybee nest – collecting water, spreading it within the brood comb, and speeding its evaporation by fanning and regurgitation – have been mentioned above. This enables the temperature in the brood area to be precisely regulated at 35°C, but humidity in the hive is less constant (Human et al., 2006). Air in the hive will generally be more humid than outside, as a result of transpiration of the inhabitants and evaporation during nectar flows. While high humidity is necessary for brood development, a dry atmosphere favours nectar ripening. We have measured absolute humidity (thus excluding temperature effects) in various regions of the hive, and found higher values in the brood area than in nectar stores, suggesting adjustments by the bees (Human et al., 2006). However, trade-offs with regulation of temperature and respiratory gases will disrupt the establishment of optimum humidity levels.

Colony level respiration is important in social homeostasis. Periodic synchronised fanning leads to tidal ventilation in honeybees and stingless bees when only one nest entrance is present (Moritz and Crewe, 1988; Southwick and Moritz, 1987). Measurement of cyclic fluctuations in water vapour pressure and temperature at the nest entrance would enable estimation of evaporative water losses at the colony level.

Excretory losses

Individual

Little is known of the excretory physiology of bees. Water balance of insects is corrected when the hindgut modifies fluid secreted by the Malpighian tubules, and in bees this may commonly mean ion reabsorption. Medium and large bees eliminate excess water as dilute urine in flight (Bertsch, 1984; Nicolson and Louw, 1982; Pasedach-Poevlele, 1940; Roubik, 1989). Diuretic hormones (DHs) are likely to be involved. The availability of the honeybee genome has enabled genomic mining for neuropeptides: using a combined bioinformatics and peptidomics approach, Hummon and colleagues have confirmed the presence of a calcitonin-like DH in *Apis mellifera* and inferred a corticotropin-releasing factor (CRF)-like DH by homology (Hummon et al., 2006). In *Bombus* and *Xylocopa* there is extensive hindgut reabsorption of Na⁺ and K⁺ from the tubule fluid, and it is clear that ion conservation is essential during diuresis (Nichol, 2000; Nicolson, 1990). The fine structure of the six elongated rectal pads of adult honeybees was described by Kummel and Zerbst-Boroffka, who also measured high osmolalities (mean 719 mosmol l⁻¹) in the pad lumen but very low osmolalities (163 mosmol l⁻¹) in the rectal lumen, showing that the rectal pads are effective in reabsorbing ions (Kummel and Zerbst-Boroffka, 1974). There are no data on the functioning of honeybee rectal pads under more dehydrating conditions necessitating water recovery from the excreta.

Colony

The non-random disposal of the excreta of social insects, such as ejecting faeces late in larval development, is assumed to be for hygienic reasons (Weiss, 2006). In honeybee larvae, the midgut–hindgut junction is occluded until the end of the larval stage, and defaecation coincides with cocoon formation, the excreta being incorporated into the structure of the cocoon (Jay, 1964). Healthy adult *Apis* do not defaecate in the nest, even during overwintering in temperate climates (but defaecation in or around the nest is a sign of infection with the midgut parasite *Nosema*).

Defaecation flights occur when weather permits, and until then rectal fluid may be stored for prolonged periods, the distended rectum occupying much of the abdominal cavity (Fig. 3). Obviously an individual honeybee's water content fluctuates enormously depending on the volume of crop or rectal contents. An inverse relationship between crop and rectal volumes has been measured in honeybees confined for varying times after feeding (Roces and Blatt, 1999).

Mass defaecation flights are conspicuous in tropical honeybees, and led to the 'yellow rain' scare, due to the high proportion of pollen exines in the faeces (Mardan and Kevan, 1989). Heat-shedding benefits have been suggested for the giant honeybee *Apis dorsata*, which builds exposed combs and engages in mass flights involving half the colony, each bee jettisoning 20% of its body mass (Mardan and Kevan, 1989). However, more comprehensive recordings for the same species show that mass flight activity is highest during maximum brood production, and timed so that brood temperature is minimally affected by the temporary absence of the protective curtain of bees (Woyke et al., 2004). Water shedding may be a more important function of mass flights than heat shedding.

Conclusion

Bees are less subject to desiccation than most terrestrial insects. This is because their nectar diet and high metabolic water production during flight frequently generate excess water. Water fluxes in the honeybee colony are also high, due to honey ripening and periodic water demand for evaporative cooling and for feeding the brood. Very importantly, the favourable microclimate created by the nest architecture and its densely aggregated inhabitants reduces evaporative water losses and provides a hydric and thermal refuge for returning foragers.

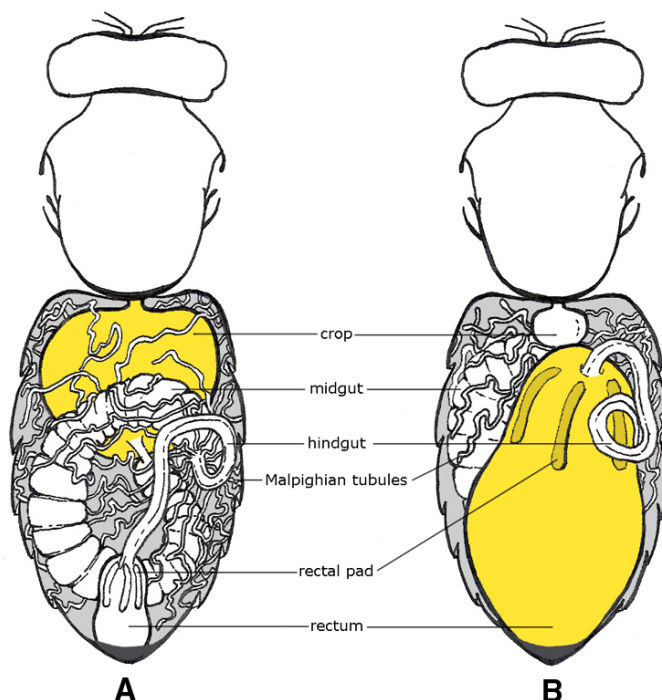


Fig. 3. Honeybee abdomen showing full crop (A) or full rectum (B). Adapted from plate 8 in Dade (Dade, 1962).

Workers of *Apis mellifera* do not forage for themselves and in social bees there is a blurring between the individual and colony in terms of water balance physiology. Common to water regulation at both the individual and colony level is the regurgitation of nectar or water on the tongue for evaporative purposes. This is involved in water elimination from nectar both in the hive and during foraging, and water is evaporated in the same way to cool either the hive or the individual bee in flight. Heinrich has previously drawn attention to the similarities in individual cooling, nest cooling and food storage behaviours (Heinrich, 1985). Perhaps these all originate in bubbling (a term I prefer to tongue lashing), which appears to function as a nectar-concentrating mechanism in a variety of bees, and may have a profound influence on the water balance physiology of solitary bees in addition to social homeostasis. The high propensity of bees for regurgitation is important in both trophallaxis, which is not confined to highly social species (Kukuk and Crozier, 1990), and bubbling to evaporate water.

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