

Bee food: the chemistry and nutritional value of nectar, pollen and mixtures of the two

Susan W. Nicolson*[‡]

Department of Zoology and Entomology, University of Pretoria, Pretoria, 0002 South Africa

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Bees are herbivorous insects, consuming nectar and pollen throughout their life cycles. This paper is a brief review of the chemistry of these two floral resources and the implications for bee nutrition. Nectar is primarily an energy source, but in addition to sugars contains various minor constituents that may, directly or indirectly, have nutritional significance. Pollen provides bees with the protein, lipids, vitamins and minerals that are essential for larval rearing. Chemical analyses of pollen have tended to focus on the protein component of bee-collected pollens as an index of nutritional quality. However, the substantial nectar content of such samples (~ 50% dry mass) should not be ignored, especially in view of current interest in measuring the nutritional quality of floral resources for bees.

Key words: bee forage, bee provisions, nectar sugars, protein, amino acids.

INTRODUCTION

Honeybees, and insect pollinators in general, are currently the focus of much scientific and media attention, owing to dramatic declines in their populations in some parts of the world, while at the same time demand is increasing for managed honeybees in agriculture (UNEP 2010). The multiple factors that may be contributing to pollinator declines include habitat fragmentation and loss, pathogens and parasites, and the use of pesticides. Interactions between these various factors are likely: for example, nutritional stress, due to a lack of floral resources or their poor quality, may lower the tolerance of bees to pesticides and diseases (Wahl & Ulm 1983; Alaux *et al.* 2010).

Nectar is the main source of energy for bees, while pollen provides proteins, lipids, vitamins and minerals for brood rearing and development. Bees, social and solitary, differ from other insects in feeding on nectar and pollen, gathered by adult females, throughout development. Oil-collecting bees use floral oil instead of nectar in their larval provisions (Steiner & Whitehead 1990) but are relatively few and will not be considered further here. In honeybees pollen consumption is highest in young adults, enabling their hypopharyngeal glands to produce jelly for feeding larvae, then after the transition to foraging at two weeks they consume mainly carbohydrate (Vasquez & Olofsson 2009; Brodschneider & Crailsheim 2010). In contrast, foraging workers eat little or no pollen,

suggesting they have reduced requirements for protein, although they have been shown to need proline for in-flight metabolism (Micheu *et al.* 2000). While the pollen requirements for reproduction of solitary bees can be substantial (Müller *et al.* 2006), it is easier to estimate the floral resources harvested by the tens of thousands in honeybee colonies. A single wild honeybee colony, for example, will harvest 120 kg of nectar and 20 kg of pollen annually in a temperate region (Seeley 1995); these amounts can be greatly exceeded in managed colonies. The need for protein during brood rearing is dramatically illustrated by the cannibalism of younger honeybee larvae that is induced by experimental pollen shortages in the colony (Schmickl & Crailsheim 2001).

This paper is a brief review of the chemistry of these floral resources, with an emphasis on South African bee forage plants, and the nutritional implications for bees. Attention is drawn to the differences in composition between hand-collected and bee-collected pollens, because honeybees, directly and indirectly, begin altering pollen composition right from the first contact.

NECTAR

The energy content of a flower's nectar depends on its volume and sugar concentration (Corbet 2003). These nectar attributes, as well as the predominant sugar types, are often included in the floral features used to characterize pollination syndromes. However, many flower visitors are not predicted by floral syndromes: an example is the winter-flowering South African bee plant *Aloe*

[‡]ZSSA Gold Medal winner for 2011.

*E-mail: swnicolson@zoology.up.ac.za

greatehdii var. *davyana*, which has the long tubular flowers and copious dilute nectar of bird-pollinated plants, yet is pollinated by honeybees and not birds (Human & Nicolson 2008; Symes *et al.* 2009).

Sugars

Bees can discriminate between small differences in nectar concentration and prefer high concentrations (Roubik & Buchmann 1984; Afik *et al.* 2006; Nicolson 2007c). Among social bees, the thoracic temperature of foragers is positively correlated with the sugar concentration in feeders (Waddington 1990; Nieh & Sánchez 2005; Nieh *et al.* 2006). However, the response of bees to the three common nectar sugars (sucrose, glucose and fructose) is less clear (Afik *et al.* 2006, and references therein). Although honeybees prefer pure sucrose to pure glucose or fructose solutions, the latter are unlikely to occur in floral nectars and mixtures are more appropriate in preference tests. In field situations, nectars rich in hexose sugars, such as those of sunflower, canola or *Eucalyptus* species, are highly attractive to bees (Neff & Simpson 1990; Nicolson 1994; Westcott & Nelson 2001). Moreover, there is considerable evidence that nectar sugar composition may be determined by plant phylogeny rather than pollinator preferences (Nicolson 2007b). For example, flowers of the Lamiaceae, a major bee-pollinated family, are characterized by nectar rich in sucrose (Petanidou 2005). Regardless of the sugar composition, nectar sugars are easily digested by honeybee sucrases (Huber & Mathison 1976). The absorption of monosaccharides has been assumed to be passive in bees (Crailsheim 1988), but recent research suggests that insects possess glucose transporters similar to those in mammals (Caccia *et al.* 2007).

While the vast majority of nectars is dominated by sucrose, glucose and fructose, the pentose sugar xylose is a major nectar sugar in *Protea* and *Faurea*, two sister genera of the Proteaceae (Nicolson & van Wyk 1998). Both *P. caffra* and *F. saligna* are important honey sources in South Africa (Johannsmeier 2001), even though honeybees are unable to taste xylose and obtain no nutritional benefit from it (Allsopp *et al.* 1998).

Nectar is a good medium for microbial growth, and contamination with yeasts, easily transferred on the mouthparts of flower visitors such as bumblebees, can reduce the proportion of sucrose in nectar (Herrera *et al.* 2008); this is also seen in older flowers (Loper *et al.* 1976). Chemical analyses of nectar in relation to pollinator types and plant

phylogeny should therefore be based on samples of freshly secreted nectar from unvisited flowers. In addition, other nectar components (see below) may confer some protection.

Water

The water content of nectar can vary greatly according to environmental conditions. Bees prefer to collect concentrated nectars, which maximize their energy gains (Roubik & Buchmann 1984), but in arid conditions they may time their foraging to maximize water gains in nectar (Willmer & Stone 1997). Dilute exposed nectars evaporate during the day and become more attractive: this applies to the nectar of *Eucalyptus* species (Nicolson 1994), which are major honey plants in South Africa and elsewhere. Alternatively, bees can deal with the excess water by evaporating it on the proboscis: honeybees collecting the dilute nectar of *A. g.* var. *davyana* (20% w/w) begin the conversion to honey on the return flight (Nicolson & Human 2008). For the more usual situation of bees collecting concentrated nectars, the water content determines the viscosity and hence the upper limit for efficient licking by bees, which is around 60% w/w sucrose (Roubik & Buchmann 1984; Harder 1986).

Other ingredients

These are present in small amounts in nectar but can be important in terms of nutrition (and contribute to the taste of honey). In addition, these compounds may have indirect effects on nectar consumption of bees by influencing nectar palatability or microbial growth. Amino acids are the next most abundant nectar solutes after sugars, although occurring in low concentrations in insect-pollinated flowers (Gardener & Gillman 2001). However, they are present in unexpectedly high concentrations in nectars of some southern African species of *Erythrina* and *Aloe*, although not in *A. g.* var. *davyana* (Nicolson 2007a). The literature on nectar amino acids is sparse, and although it has been suggested that pollen contamination might be responsible for some of the measured values, I was able to rule this out in nectar of *A. marlothii*. Proline in nectar has been claimed to attract honeybees (Carter *et al.* 2006); glycine is also attractive and improves the learning performance of bees (Kim & Smith 2000). When we tested the responses of honeybees to added amino acids, using the proboscis extension reflex, proline, glycine and phenylalanine at concentrations of

2 mM all elicited positive responses in 5% w/w sucrose (C.W.W. Pirk *et al.*, unpubl. data). Proline is the most abundant amino acid in honey and its analysis is used in quality control (Hermosin *et al.* 2003): the high concentrations are derived from glandular secretions added during regurgitation as bees process the nectar into honey.

Little is known of inorganic ion concentrations in nectar (Nicolson 2007b), but high K⁺ levels in onion and avocado nectar are thought to be unattractive to honeybees (Waller *et al.* 1972; Afik *et al.* 2006). Salts in nectar (and pollen) may be important in osmoregulation of bees (Nicolson 1990).

Secondary metabolites in nectar, such as alkaloids and phenolics, are paradoxical in view of the attractive function of nectar: however, they may serve to attract specialized pollinators or deter unwanted visitors (Adler 2000). Recently we have examined the responses of honeybees to nicotine as a model alkaloid, and found that deterrence depends on the nicotine concentration but also on the sugar concentration (A. Köhler *et al.*, unpubl. data). Caffeine is present in *Citrus* nectar, which is highly attractive to bees (Kretschmar & Baumann 1999). Phenolics in the nectar of *A. vryheidensis* deter honeybees (Johnson *et al.* 2006), but the lower phenolic levels in nectars of other *Aloe* species are less deterrent (S. Johnson, pers. comm.). In other studies phenolics have been reported as attractive to bees (Hagler & Buchmann 1993; Afik *et al.* 2006; Liu *et al.* 2007). Some nectar toxins are not detected by bees, such as amygdalin in almond nectar, but postingestive effects contribute to avoidance learning (London-Shafir *et al.* 2003; Wright *et al.* 2010). One possible benefit of these secondary metabolites in nectar may be antibiotic activity against microbes (Adler 2000), a function that is shared by proteins in nectar, termed nectarins (Thornburg *et al.* 2003).

POLLEN

Most chemical analyses of pollen consist of protein determinations on bee-collected pollen obtained from pollen traps (for entomophilous plants, this is much easier than collecting fresh pollen). When honeybees pack pollen into their corbiculae, the addition of nectar enables them to transport pollen grains varying in size and surface texture (Thorp 1979). More nectar and glandular secretions are added in the hive, and microbial inoculation leads to fermentation during storage, forming bee bread which has higher nutritive value than bee-collected pollen (Herbert & Shimanuki 1978;

Vasquez & Olofsson 2009; Brodschneider & Crailsheim 2010). Lactic acid bacteria are already present in bee-collected pollen, and may be a source of vitamins (Vasquez & Olofsson 2009). The nutritional chemistry of hand-collected, bee-collected and stored pollen has been compared for two important South African bee plants: *A. g.* var. *davyana* (Human & Nicolson 2006) and sunflower *Helianthus annuus* (S.W. Nicolson & H. Human, unpubl. data).

Protein

Protein levels in pollen vary widely, from 2–60% dry mass in the hand-collected pollens of 377 species analysed by Roulston *et al.* (2000). Interestingly, these authors found no evidence that pollen protein concentration is higher in bee-pollinated species, but did demonstrate a relationship with plant phylogeny. Hand-collected pollen of *A. g.* var. *davyana* has a high protein concentration of 51% dry mass, but this is diluted to 31% during collection by honeybees and to 28% in bee bread (Human & Nicolson 2006). Sunflower pollen is considered to be of poor quality for bees because of its low protein content of 13–15% after collection by bees (Pernal & Currie 2000; Somerville & Nicol 2006; S.W. Nicolson & H. Human, unpubl. data). There is little evidence that bees can assess pollen quality and choose pollens with high protein levels (Pernal & Currie 2001; Roulston & Cane 2002). However, manipulation of the protein content of pollen provided to bumblebee colonies led to a small but significant increase in thoracic temperatures of bees collecting higher quality pollen (Mapalad *et al.* 2008). Cook *et al.* (2003) showed that bees provided with two pollens chose the one that was richer in some essential amino acids, but other factors may have been involved and more studies are needed. Pollen substitutes developed for the supplementary feeding of honeybee colonies are based on high protein levels (Brodschneider & Crailsheim 2010). However, our study of protein and carbohydrate regulation in caged worker honeybees shows that high protein may not always be beneficial in the absence of larval rearing (Altaye *et al.* 2010); see also Roulston & Cane (2002); Human *et al.* (2007).

Amino acid composition determines the amount of pollen required by bees, more than its protein content. The minimum amino acid requirements for honeybees were established by de Groot (1953); these can be compared with data on the amino acid composition of various pollens, usually

Table 1. Proportions of pollen and nectar in bee provisions.

Species	Protein in hand-collected pollen (% dry mass)	Protein in bee provisions* (% dry mass)	Fraction of dry mass that is pollen**	Reference
<i>Virgilia divaricata</i>	25.0 ± 2.0	15.9 ± 0.9	0.64	Louw & Nicolson 1983
<i>Aloe greatheadii</i> var. <i>davyana</i>	50.8 ± 2.7	28.1 ± 1.6	0.55	Human & Nicolson 2006
<i>Helianthus annuus</i>	26.5 ± 0.6	13.3 ± 0.1	0.50	Nicolson & Human, unpubl.

Protein concentrations given as means ± S.E. (*n* = 6)

*Brood provisions of carpenter bees (*Xylocopa capitata*) foraging on *Virgilia divaricata* or stored pollen collected by *Apis mellifera scutellata* from aloes or sunflowers.

**Assumes no protein contributed by nectar.

bee-collected pollens in which a nectar or glandular origin of amino acids cannot be ruled out (Loper & Cohen 1987; Cook *et al.* 2003; Human & Nicolson 2006; Somerville & Nicol 2006). Bee-collected pollens of *Eucalyptus* species have a protein concentration of 24.9 ± 0.6% dry mass (mean ± S.E., *n* = 19 species), but are generally deficient in isoleucine (Somerville & Nicol 2006), suggesting that bees will not do well on an exclusive diet of *Eucalyptus* pollen. Poor brood rearing in bees fed dandelion pollen may be due to inadequate levels of several amino acids (Loper & Cohen 1987).

Carbohydrate (and the mixture problem)

In stored aloe and sunflower pollen, the added nectar and secretions cause other nutrients such as protein to be diluted by ~50% (Table 1). The added nectar will mainly affect the carbohydrate component of the brood food, but possibly other components too, for example if amino acid levels are high in the nectar being collected. The data on pollen composition reported by Todd & Bretherick (1942) included a single species, *Pinus contorta*, represented in both hand-collected and bee-collected samples: the percentage of sugars increased by 34% in bee-collected pollen.

It is seldom acknowledged that nectar sugars can be a substantial component of pollen pellets. A notable exception is the extensive data set on pollen protein concentrations of Roulston *et al.* (2000) which is based on hand-collected pollen. For nine plant species, these authors compared protein concentrations in hand-collected and bee-collected pollens, and found a pollen fraction of 0.57 of the total mass (range 0.38–0.83): although the samples of hand-collected and bee-collected pollens were not collected at the same time (T. Roulston, pers. comm.). Similar protein concentrations have been reported in hand-collected and bee-collected

almond pollen, but the former was obtained by sifting shredded anthers through copper mesh (Loper *et al.* 1980; Standifer *et al.* 1980), and may have been contaminated with anther tissue which would be low in protein; see higher values for hand-collected pollen of other *Prunus* species in Roulston *et al.* (2000). Some researchers have washed bee-collected pollens to remove the sugars and enable comparison with hand-collected pollens (Müller *et al.* 2006, Hanley *et al.* 2008); this method is suitable for counting pollen grains but not for chemical analyses, because it risks the loss of other components and has not been tested against hand collected pollen. Scott *et al.* (1993) used ethanol extraction to separate the sugars from carpenter bee provisions, and found a sugar content of 4–69% dry mass.

Starch is absent from stored pollen, no doubt due to enzymatic action (Herbert & Shimanuki 1978). Fibre is not commonly analysed in pollen but makes up 7% dry mass in hand-collected *Virgilia* pollen (Louw & Nicolson 1983) and 7% on average in the bee-collected samples of unknown floral origin analysed by Herbert & Shimanuki (1978). Fibre may represent a higher proportion of total mass in small pollen grains. Because a high proportion of pollen-derived carbohydrate may be indigestible, the added nectar sugars are nutritionally important to bees.

Other ingredients

Total lipid content is usually less than 10% dry mass. Low lipid levels (0.6–1.9% dry mass) are found in bee-collected pollen of eucalypts (Bell *et al.* 1983; Manning & Harvey 2002), whereas an extraordinary value of 32% dry mass is given for canola pollen by (Evans *et al.* 1987). Higher levels of lipid are considered attractive to bees (Singh *et al.* 1999).

Fatty acid composition is known for some pollens, and may be nutritionally important (Manning 2001). Some fatty acids show antibiotic activity against the causative agent of American foulbrood disease (Feldlaufer *et al.* 1993). Pollenkitt, the sticky coating on entomophilous pollens, contains fatty acids, and volatile components may be chemical cues for pollinators (Dobson 1988) rather than of nutritional value. Sterols in pollen are essential for bees (Brodschneider & Crailsheim 2010).

As in the case of nectar, it is not clear whether secondary metabolites are present because of defensive roles in the rest of the plant. High concentrations of nicotine and amygdalin have been measured in hand-collected pollen of tobacco and bee-collected pollen of almond, respectively (Detzel & Wink 1993, London-Shafir *et al.* 2003). For plants containing pyrrolizidine alkaloids, high values in pollen may lead to contamination of nectar and then honey (Boppré *et al.* 2005; Reinhard *et al.* 2009).

CONCLUSION

Pollen protein levels are frequently underestimated in the literature because the information is derived from bee-collected pollens containing an unknown amount of added nectar. Bee-collected pollen pellets of *Echium plantagineum*, an important introduced forage plant in Australia, contain 37.4% protein (Corbet & Delfosse 1984; Somerville & Nicol 2006) – does this reflect protein levels exceeding 70% dry mass in fresh pollen of this species, or do honeybees add less nectar to *E. plantagineum* pollen during collection? The extent of dilution with nectar sugars is not predictable: it may depend on the properties of the pollen or the concentration of nectars available to bees at the time (Roulston & Cane 2000).

The availability of pollen nutrients to consumers depends on how easily the pollen can be digested, and it is possible that added sugars may enhance this process. The pollen exine is generally considered the main barrier to digestion, but extensive extraction of pollen grain contents can take place through pores while the exine remains intact (Human & Nicolson 2003; Human *et al.* 2007). Various possible methods used to digest pollen are detailed by Roulston & Cane (2000), who point out the confusing data obtained from different plant and bee species. Studies comparing the digestion of fresh pollen and food provisions by bees would show whether the addition of nectar sugars aids

digestion of pollen grains (Roulston & Cane 2000). Pollen digestion is not a problem for honeybee larvae because of the transformation to protein-rich jelly in the hypopharyngeal glands of nurse bees. The relative proportions of pollen and nectar in bee provisions are thus of more direct relevance to the larvae of mass-provisioning species.

It is commonly assumed that bees visit more plant species for nectar than for pollen, and the concept of floral specialization in bees refers to pollen foraging. Cane & Sipes (2006) discuss various methods used to determine pollen use by bees, and point out the need to consider widely differing pollen grain volumes when quantifying bee diets. Nectar sources, however, can be determined only by direct observation of foragers at flowers: the examination of pollen grains in honey is not a reliable method (Todd & Vansell 1942). From an evolutionary viewpoint it makes sense for bees to be more discriminating in their choice of pollen sources: the pollen is needed on a short-term basis for larval rearing, whereas the colony requires full honey stores to survive adverse conditions. Diversity in bee diets may be necessary to avoid nutritional deficiencies (Schmidt *et al.* 1995) and to dilute toxins, but need not involve many plant species. Honeybee colonies sampled at various sites in Israel over a year usually collected pollen from 5–9 species at a time (Avni *et al.* 2009). Similar dependence on a few pollen sources was noted by Keller *et al.* (2005), based mainly on Swiss studies, and mixed pollens from five plant species were found to be better than single pollens for maintaining honeybee immune systems (Alaux *et al.* 2010). More data on the amino acid composition of fresh pollens are needed to assess the nutritional significance of these pollen choices at the colony level: are they due to floral constancy or the selection of complementary pollens to compensate for amino acid imbalances?

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