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FORUM ARTICLE

Taste perception in honeybees: just a taste of honey?

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Abstract The advent of the genomic era has opened new doors to understand the fundamental organization of living organisms and has therefore promoted a fertile field of comparative research that intends to identify similarities and differences between related and unrelated species at the genomic level. One of the organisms whose genome has been recently decoded is that of the honeybee *Apis mellifera*, enabling a direct comparison with another well-studied insect, the fruit fly *Drosophila melanogaster*. It was reported that the honeybee has only ten gustatory receptors and thus a very poor taste perception compared to *Drosophila*, which presents 68 gustatory receptors, and the mosquito *Anopheles gambiae*, which presents 76 gustatory receptors. In this forum article, we discuss the implications of these findings taking into account previous and new discoveries on honeybee gustation based on behavioral and neurobiological studies by several authors and us. We conclude that the world of taste of a honeybee might not be as poor as proposed and that further studies should integrate molecular, neurobiological, behavioral and ecological approaches to better characterize taste perception in bees.

Keywords *Apis mellifera* · Gustation · Gustatory receptors · Honeybee · Taste

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Introduction

Since the decoding and publication of the genome of the honeybee *Apis mellifera* (The Honeybee Genome Sequencing Consortium 2006), researchers interested in different aspects of the biology of the honeybee have access to bioinformatics tools that allow performing comparative research using as a model the other insect for which most is known in terms of genetic architecture, the fruit fly *Drosophila melanogaster*. In this way, it is possible to search for similarities and differences at the genomic level in order to understand functional principles of the bee biology. Although the value of the comparison between fruit flies and honeybees is relative to the absence of genomic information for other hymenopterans or even other primarily nectar-feeding holometabolous insects, no other comparison with more closely related or ecologically similar insects is available for the moment.

Such a cross-comparative analysis has been used recently to characterize the olfactory and gustatory receptors of honeybees (Robertson and Wanner 2006). The characterization of olfactory receptors yielded expected results based on the notion that the number of olfactory receptors coincide with the number of glomeruli, which are the functional units of the antennal lobe, the primary olfactory center of insects. Indeed, the number of olfactory receptors identified in the honeybee genome is approximately equal to the number of glomeruli in the bee antennal lobe (160–170; Galizia and Menzel 2001). However, the same study reported unexpected results in the case of gustatory receptors (Robertson and Wanner 2006). Only ten gustatory receptors were reported so that the gustatory world of bees was described as being very limited.

This description contrasts, in principle, with the variety of natural gustatory compounds to which bees are exposed in different activities of their normal life (Winston 1987). In a foraging context, for instance, bees collect nectar and pollen, which provide carbohydrates and proteins, respectively, necessary for survival. Nectar presents not only different types of sugars such as sucrose, glucose and/or fructose but also organic acids, lipids, minerals, vitamins and aromatic compounds, even if these substances constitute a low percentage of nectar contents. Pollen contains proteins but also lipids, mineral salts, albumin, vitamins, amino acids, growth regulator factors, folic acid and enzymes among others. Thus, characterizing the gustatory world of bees as limited is surprising in the light of these and other ecological aspects of the gustatory biology of bees.

Moreover, having ten gustatory receptors does not necessarily imply an impoverished perceptual world as perceptual richness can be built with relatively few input channels. Humans can see over a million colors with only three color receptor types based on defined combinations or ratios of receptor signals. In fact, more receptor types do not necessarily increase the dimensionality of perception as they might be linked in parallel to motor pattern generators, rather than being processed in a multidimensional perception space. The simultaneous excitation of few taste receptors might generate different perceptual sensations at the central level, thus creating a rich taste world.

In this forum article, we challenge the hypothesis that posits that the gustatory world of bees is limited and discuss findings by several authors and us, which suggest that the sense of taste of honeybees might not be as poor as proposed. We conclude that further studies should integrate molecular, neurobiological, behavioral and ecological approaches to better characterize taste perception in bees.

The biological basis of taste perception in bees

Although the processing of sensory information of flowers (e.g. colors, odors) by honeybees has been intensively studied in the last decades (Chittka and Brockmann 2005; Chittka and Raine 2006), less is known about the processing of gustatory stimuli that are perceived when bees contact different natural products such as nectar and pollen. Although there has been a fair bit of research on the behavioral aspects of gustatory responses (Scheiner et al. 2001, 2005; Scheiner 2004), honeybee taste, contrarily to other sensory modalities, remains a mainly unexplored research field.

In the honeybee, the antennae, mouth parts and tarsi of the forelegs constitute the main chemosensory organs (Goodman 2003; see Fig. 1a). They include gustatory but also hygro, thermo, mechanosensory and olfactory receptors. Gustatory receptor cells on these structures are located within specialized cuticular structures called sensillae (Fig. 1b), which often take the form of hairs (chaetic sensillae) or pegs (basiconic sensillae) (Esslen and Kaissling

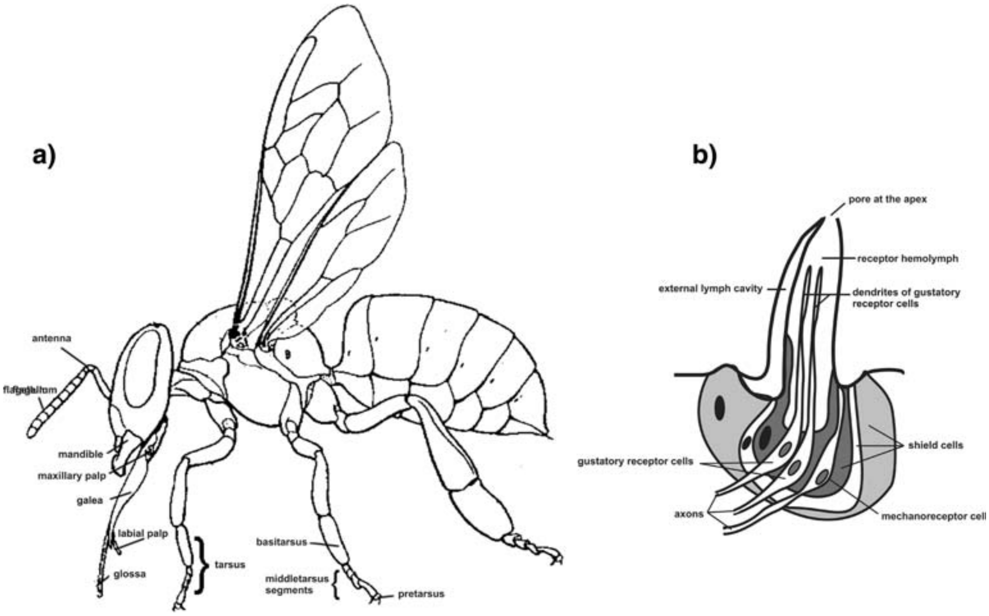

Fig. 1 (**a**) Anatomy of the honeybee. The main chemosensory organs involved in taste perception (antennae, mouth parts and tarsal regions of the legs) are indicated. (**b**) Structure of a taste sensilla in longitudinal section. The sensilla consists of a cuticular evagination presenting a pore at its apex through which gustatory substances may penetrate. Two gustatory neurons, with different gustatory specializations, are shown, together with a mechanoreceptor neuron. The sensilla cavity is closed by shield cells and contains a receptor hemolymph, which is separated from the hemolymph circulating in the rest of the body

1976). These sensillae have a characteristic aperture at the apex through which gustatory substances can penetrate after contacting the hair or peg (contrarily to olfactory sensillae whose walls are covered by tiny pores which allow the diffusion of olfactory molecules). Gustatory receptor cells innervate each sensilla and bath in a receptor hemolymph. Each neuron projects a dendritic branch up the shaft of the hair or peg to the apex. Such a branch—and there may be 3–5 per sensilla, corresponding to 3–5 gustatory receptor neurons (Dostal 1958)—bears the molecular receptors to which a gustatory substance will bind if it is the appropriate molecule for these receptors. Such receptors are G-protein coupled proteins. In some cases, a mechanoreceptor cell terminating at the base of the shaft can also be found. This neuron is stimulated not by gustatory stimuli but by movement of the sensilla. As gustatory organs have to explore and manipulate food, evaluating the position and density of the food is facilitated by the presence of mechanoreceptor cells associated with gustatory receptor cells within the same sensilla. Gustatory molecules arrive to the molecular receptors on the membrane of the gustatory neuron though passive diffusion in the hemolymph of the sensilla, or through active transport by carrier proteins. When a gustatory molecule binds to a molecular receptor, the receptor cell depolarizes and, if the magnitude of the receptor potential is enough, an action potential is generated. The gustatory message is then relayed to the central nervous system where it is processed in the subesophageal ganglion.

Taste sensillae can be found essentially on the antennae, mouthparts and legs of a honeybee (Whitehead and Larsen 1976a) (Fig. 1). On the antennae, gustatory sensillae can be localized on the segments that constitute the flagellum and on the tip. On the mouthparts, they can be localized on the proboscis (formed by the maxillae and the labium), the glossa, the labial palps and the galeae of the maxilla. On the legs, they have been reported on the tarsus and pretarsus of the forelegs. The tarsi of the mid and hindlegs also bear taste sensillae but these have been poorly characterized (Frings and Frings 1949). So far, research on gustatory function in bees has focused on these body appendages. However, gustatory receptors could be situated in unsuspected parts of the body. For instance, such receptors could be found in the oral cavity and in the crop. Such internal receptors could act as molecule counters allowing the estimation of the intake rate of gustatory stimuli such as sucrose. In this case, they would allow a concrete estimation of food source profitability.

Gustatory sensillae play an important role in appetitive food sensing as shown by the fact that stimulation of antennae, tarsi and mouth parts with sucrose solution elicits the so-called proboscis extension reflex (PER) (Takeda 1961; Bitterman et al. 1983). Gustatory sweet receptors on the antennae are more sensitive than those of the legs as shown by the fact that bees extend the proboscis to sucrose solution at a threshold concentration of 2.85% if applied to the antennae, and of 34.23% if applied to the tarsi (Marshall 1935). At the central level, a unique neuron, VUMmx1 (initials of Ventral Unpaired Median maxillar1 neuron) whose cell body can be found in the maxillary neuromere of the subesophageal ganglion (Fig. 2), responds to stimulation with sucrose solution to the antennae. The subesophageal ganglion is a fused neuropile consisting of the labial, maxillary and mandibular neuromere. The neural activity of this neuron substitutes for sucrose in olfactory learning (Hammer 1993). In other words, pairing of an odorant with an artificial depolarization of VUMmx1 generated by injecting current into the neuron is the equivalent of having experienced an odorant followed by sucrose. As a consequence, a bee treated in this way learns to respond with a PER to the odorant even if it had never experienced real sucrose associated to it. How gustatory sucrose receptors convey information to VUMmx1 is still unknown but it is thought that they project to the subesophageal ganglion where they would synapse directly or indirectly onto VUMmx1 (Fig. 2). Recently, a novel neuron, VUMmd1 (Ventral Unpaired Median mandibular 1 neuron, whose cell body can be found in the mandibular neuromere), was found, which presents a morphology similar to that of VUMmx1 neurons and which also responds to sucrose stimulation of the antennae (Schröter et al. 2007). Thus encoding of sucrose taste could be performed by these neurons at the central level. However, VUMmx1 neurons as recorded by Schröter et al. (2007) also responded sometimes to water and salt thus making the question of taste encoding in the bee brain even more complex.

Uncovering functional aspects of honeybee taste

Previous electrophysiological studies on honeybee gustatory receptors have essentially focused on sensillae located on the mouth parts associated with the proboscis, such as the galea (Whitehead and Larsen 1976a, b) and the palps (Whitehead 1978). Cells with different sensitivities to sugars (sucrose, fructose, glucose) and salts (NaCl, KCl, LiCl) were reported there. Haupt (2004) studied the electrophysiological responses to sucrose solution of taste sensilla on the tip of the antennae of the honeybee. This choice was justified by previous findings showing that taste receptors are more concentrated in this part of the antennae (Esslen and Kaissling 1976). Haupt (2004) reported the existence of taste sensilla which responded to sucrose concentrations down to 0.1% and which exhibited highly variable spike frequencies. Such variability was suggested to be a mechanism to extend the dynamic range of sucrose perception over a broad spectrum of concentrations. From these recordings, it could

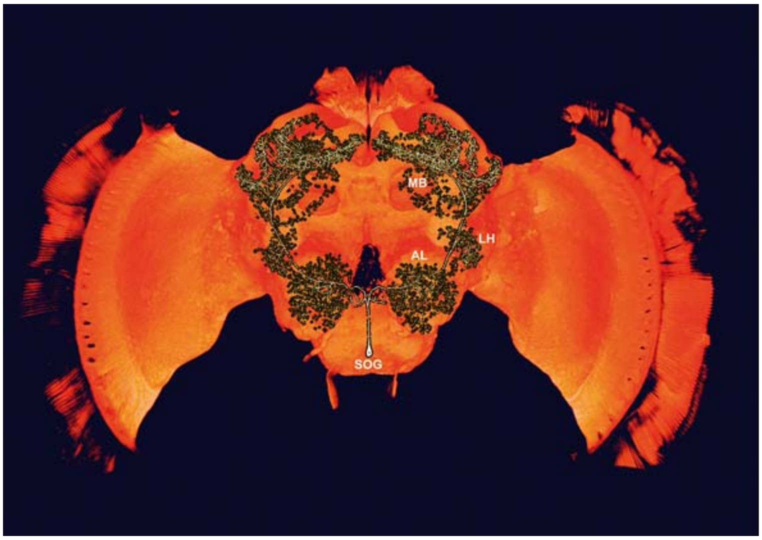

Fig. 2 Frontal section of a honeybee brain showing the localization of the VUMmx1 neuron, whose activity substitutes for the reward of sucrose solution in associative olfactory conditioning in honeybees. The neuron contacts the main structures of the olfactory circuit, the antennal lobes (AL), lateral horns (LH) and mushroom bodies (MBs). Its cellular body can be found in the subesophageal ganglion (SOG) at the level of the maxillar neuromere

not be discerned how many gustatory receptors were activated within each of such taste sensilla, nor what their sensitivity was (Haupt 2004). It seems, however, that all taste sensillae recorded at the antennal tip possess at least one receptor neuron activated by sugar and no receptor neuron activated by water (Haupt 2004).

The question of whether bees perceive bitter substances has received little attention so far. Von Frisch (1967) reported that honeybees are insensitive to bitter substances. However, Chittka et al. (2003) reported that bumble bees reacted to quinine stimulation which seems to be aversive and to improve the learning of visual discriminations in which one color is rewarded with sucrose solution and another color is penalized with quinine solution (Chittka et al. 2003). Bumblebees also develop a strong aversion to flowers containing an alkaloid but this aversion is only expressed when alternative flowers with lower levels of nectar alkaloids are made available (Gegear et al. 2007).

Asking about bitter taste in bees is relevant because bees are confronted with such substances in their natural surroundings. Flower nectar may contain aminoacids, phenolics, alkaloids and other secondary compounds such as nicotine and caffeine (Liu et al 2004, 2007; Singaravelan et al. 2005), which may be perceived as bitter (see below). The fruit fly *D. melanogaster* possesses bitter receptors, which is ecologically useful since it may often encounter food sources rich in nutrients but contaminated with toxic chemicals derived, for instance, from substrate fermentation. The fly has, therefore, to choose between feeding and avoidance, depending on the impact the particular toxic compound may have on its health. Bitter receptors of *D. melanogaster* have been recently characterized at the molecular level (Moon et al. 2006), thus opening perspectives for the use of bitter gustatory stimuli to study aversive learning.

In the case of honeybees, only one study has examined whether or not bitter taste perception occurs at the level of the antennae, both at the behavioral and the electrophysiological levels (de Brito Sanchez et al. 2005). It was shown that neither quinine nor salicin delivered at one antenna at different concentrations induced a retraction of the proboscis once it was extended in response to sucrose solution delivered to the opposite antenna. Bees that extended massively their proboscis to sucrose 1 M responded only partially when stimulated with a mixture of sucrose 1 M and quinine 100 mM. The mixture of sucrose 1 M and salicin 100 mM had no such suppressive effect. No behavioral suppression was found for mixtures of salt solution and either bitter substance. Electrophysiological recordings of taste sensillae at the antennal tip revealed sensillae that responded specifically either to sucrose or salt solutions, but none responded to the bitter substances quinine and salicin at the different concentrations tested. The electrophysiological responses of sensillae to sucrose solution 15 mM were inhibited by a mixture of sucrose 15 mM and quinine 0.1 mM, but not by a mixture of sucrose 15 mM and salicin 0.1 mM. The responses of sensillae to NaCl 50 mM were reduced by a mixture of NaCl 50 mM and quinine 1 mM but not by a mixture of NaCl 50 mM and salicin 1 mM. It was therefore concluded that no receptor cells for the bitter substances tested exist at the antennal tip of the honeybee and that antennal bitter taste is not represented as a separate perceptual quality (de Brito Sanchez et al. 2005). Nevertheless, the fact that quinine inhibits both behavioral and electrophysiological responses when mixed with sucrose suggests that it has a perceptual effect *per se* at the level of the antennae. Similar studies performed at the level of the mouth parts and the tarsi are necessary and are currently under way. Dedicated bitter receptors, if any, might be located in other regions of the body.

Behavioral and electrophysiological experiments as described above have yielded only partial answers to the question of which gustatory stimuli are relevant for honeybees. It seems clear that bees taste sucrose, fructose and glucose (Whitehead and Larsen 1976a, b) but we have no certitudes about the number of molecular receptors involved in sweet taste perception and their specificity. Similarly, it appears that bees can taste some salts but we still do not know whether one or more molecular receptors are required to this end. Additionally, we don't know whether bees, like *Drosophila*, can taste bitter (Moon et al. 2006) and/or spicy compounds (Al-Anzi et al. 2006). In this context, using bioinformatics tools to search for gustatory genes similar to those that have been already characterized in the fruit fly genome is a promising procedure that has been recently applied to the honeybee (Robertson and Wanner 2006). The basic principle of this procedure consists in the establishment of the correspondence between genes (orthology analysis) or other genomic features in different organisms. Specific mathematical and probabilistic models and algorithms are employed to detect homologies and compute protein families in a comparative way. In this way, the presence of candidate gustatory receptor genes can be identified in a faster way, thus allowing further studies aiming at localizing these receptor genes in the honeybee body (for instance, by means of quantitative RT-PCR and/or in situ hybridization) and characterizing their gustatory tuning (for instance, by means of electrophysiology).

The advent of molecular studies on honeybee gustation

As mentioned in the Introduction, a recent publication reported a first characterization of gustatory receptors in honeybees by means of bioinformatics tools and quantitative RT-PCR using the *Drosophila* genome as a reference (Robertson and Wanner 2006). In the fruit fly *Drosophila melanogaster* 68 gustatory receptors encoded by 60 genes through alternative splicing have been identified (Dunipace et al. 2001; Scott et al. 2001; Robertson et al. 2003; Amrein and Thorne 2005; Scott 2005). Some of these gustatory receptors have been linked to specific gustatory stimuli. For instance, DmGR5a has been associated with sweet taste as it responds specifically to trehalose (Ueno et al. 2001; Dahanukar et al. 2001; Chyb et al. 2003; Marella et al 2006). DmGr66a, on the other hand, has been associated with bitter taste as it responds to caffeine (Marella et al. 2006; Moon et al. 2006). Other studies have attributed gustatory receptor neurons to specific gustatory stimuli without being able to characterize the molecular receptor involved in such perception. For instance, electrophysiological experiments performed on a sensilla type of the labellum of the fruit fly, the i-type, showed that one of the two neurons present in this sensilla responds to bitter compounds among which strychnine is the most potent; and also to salt at high concentrations (over 400 mM NaCl) while the other neuron responds to sugar and to low concentrations of salt (10–50 mM NaCl) (Hiroi et al. 2004).

Bioinformatic identification of gustatory receptor genes in the honeybee genome taking as reference the *Drosophila* genome yielded a surprising result: only ten intact gustatory receptor genes were found, which was taken as a proof of a rather limited taste repertoire, at least compared to that of fruit flies (Robertson and Wanner 2006) and mosquitoes (76 gustatory receptors encoded by 52 genes; Hill et al. 2002). From these ten gustatory receptor genes, two (AmGr1 and AmGr2) seem to correspond to the eight candidate sugar receptors identified in the fly, based on the role of DmGr5a as a trehalose receptor (see Chyb et al. 2003). The specificity of the other eight remains to be determined.

The explanation provided by Robertson and Wanner (2006) to account for such a limited number of gustatory receptor genes mentions that bees have little need for gustatory receptors to locate and recognize food because flowering plants have evolved mechanisms to attract and reward bees for pollination services. They argue, in addition, that bees do not require the ability to detect and discriminate between the numerous plant secondary chemicals and toxins usually deployed in the chemical ecological arms races between most plants and many insect herbivores so that there is no need for the bees to develop additional taste receptors. Several additional explanations, other than the one offered by Robertson and Wanner (2006), could be provided to account for the difference in the number of receptor orthologues identified in the honeybee genome from comparison with fruit flies. One hypothesis for why fruit flies and mosquitoes have more gustatory receptors is phylogenetic and posits that their common dipteran ancestor may have undergone gene duplication for several receptors. The odorant binding protein and chemosensory protein gene families have very different histories in the Diptera, Lepidoptera and Hymenoptera (Hallem et al. 2006). Another hypothesis is functional; fruit flies regularly assess the degree of substrate fermentation as well as sugar meals so that they may need to track more diverse gustatory stimulants than most hymenopterans.

A limited taste repertoire in honeybees?

The arguments mentioned above could be questioned along several lines. Firstly, the study of Robertson and Wanner (2006) does not consider that a same gustatory receptor gene may encode for different receptor proteins through alternative splicing, thus enhancing considerably the gustatory repertoire of an organism. In other words, although ten gustatory receptor genes have been characterized, these may in fact encode more than just ten molecular receptors. In particular, we suggest that the two gustatory receptor genes which have been attributed to sweet taste (AmGr1 and AmGr2) may have in fact several splicing forms, which could relate to the bees' capacity to respond electrophysiologically to different kinds of sugar such as sucrose, fructose and glucose (Whitehead and Larsen 1976a, b; Whitehead 1978).

Secondly, as mentioned in the Introduction, having ten gustatory receptors does not necessarily imply an impoverished perceptual world as perceptual richness can be built with relatively few input channels. Studying the central coding of gustatory substances at the level of the subesophageal ganglion of the honeybee is therefore crucial to determine whether the simultaneous excitation of few taste receptors generates a complex and rich pattern of taste perceptual sensations.

Thirdly, the expression patterns of nine of the ten gustatory receptor genes reported by Robertson and Wanner (2006) are intriguing (Fig. 3). Expression was measured through real-time quantitative PCR in the head, the glossa and the antennae. Other regions of the body that have been consistently associated with taste in bees (e.g. the tarsi; see Goodman 2003) were not included in the analyses. Also, AmGr1 and AmGr2, the sweet receptors that should be abundantly expressed following Robertson and Wanner's arguments on the kind of relationship that bees have developed with plants, are barely expressed in the body parts where they should be definitely present (antennae and glossa, for instance). Other genes, whose specificity is currently unknown (e.g. AmGr4 and AmGr7), are expressed 5–10 times more in the mouth parts, thus raising questions about their specificity.

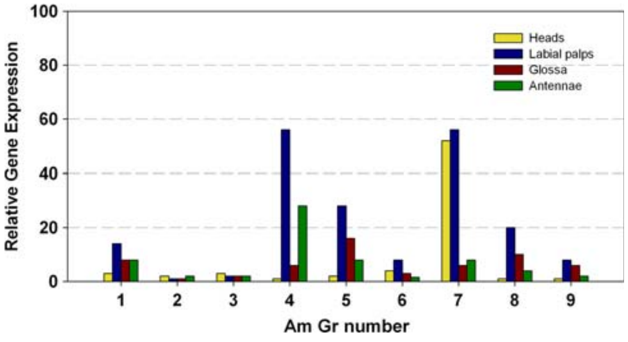

Fig. 3 Gustatory gene (AmGr) expression in the head, labial palps, glossa and antennae of honeybees. Expression was measured as fold increases relative to body levels. Real-time quantitative PCR was used to determine gene expression levels which were normalized to levels of a ribosomal protein S8 found in honeybees (adapted from Robertson and Wanner 2006). AmGr1 and AmGr2 have been related to the eight candidate sugar receptors identified in the fly, based on the role of DmGr5a as a trehalose receptor (see Chyb et al. 2003)

Fourthly, the biology of taste of honeybees is certainly much more complex than what was suggested by Robertson and Wanner (2006). Foraging for nectar does not only rely on detecting different levels of sugars such as sucrose, glucose and/or fructose in order to assess food source profitability but also exposes the bees to other components present in nectars such as organic acids, lipids, minerals, vitamins and aromatic compounds, even if these substances constitute a low percentage of nectar contents (Winston 1987). Furthermore, the argument proposed by Robertson and Wanner (2006) to justify the scarceness of bee gustatory receptor genes, stating that bees do not have the ability to detect and discriminate between the numerous plant secondary chemicals and toxins usually employed as defense by some plants, is unjustified in the light of new findings on the responses of foraging bees to nectar (and pollen) with high contents of phenolic compounds and other secondary compounds such as nicotine and caffeine (Liu et al. 2004, 2007; Singaravelan et al. 2005). Naturally occurring plants such as *Nicotiana* sp., *Citrus* spp. and A*mygdalus* spp., which contain various alkaloids in their nectars, completely depend on bees for pollination (Detzel and Wink 1993; Kretschmar and Baumann 1999; London-Shafir et al. 2003). Recent studies show that these compounds interact with nectar sugar to increase honey bees foraging (Liu et al. 2004; Singaravelan et al. 2005). It has been proposed that synergism between phenolics and sugar in nectar may provide a novel mechanism for plants to encourage pollinating bees and reduce energy investment in nectar, operating as exaptations by co-opting defense mechanisms against herbivores (Liu et al. 2007). In other words, bees should be able to taste the presence of these different secondary compounds in nectars in order to improve their foraging efficiency. Similar arguments apply to pollen which presents proteins, lipids, mineral salts, albumin, vitamins, amino acids, growth regulator factors, folic acid and enzymes among others. Here again, secondary compounds such as phenolics play an essential role in the context of bee foraging activities. It has been shown that honeybee foragers detect and estimate the amount of phenolics in pollen so that pollen-foraging activities of a honeybee colony are regulated by quantitative changes in phenolic contents of pollen. Honeybees seem, therefore, to use non-nutritional factors, such as pollen phenolics, to assess colony requirements and to change foraging dynamics accordingly (Liu et al. 2006).

Besides foraging for nectar and pollen, bees collect water and in this context they respond to salts and indeed electrophysiological responses to salts (NaCl, KCl, LiCl) have been measured (Whitehead and Larsen 1976a, b; de

Brito Sanchez et al. 2005) both at the level of the mouth parts and antennae. Additionally, bees collect resin for elaborating propolis and should then taste several compounds such as prenylated and non-prenylated phenylpropanoids, terpenoids and anthracene derivatives, which have been identified in the resin loads transported in the corbiculae (Weinstein Texeira et al. 2005). Finally, bees chew and process wax with their mouth parts. Although it has been reported that contact chemoreception is not necessary for detecting the presence of wax (Brockmann et al. 2003), one should not confuse detecting the airborne components of wax and tasting the chemicals in it.

Conclusion

The examples mentioned above argue in favor of a gustatory receptor repertoire that could be more complex than what has been proposed so far (Robertson and Wanner 2006). Clearly, only ten gustatory receptor genes may exist in the bee genome but research should elucidate whether or not these genes give origin to different splicing forms and thus to different receptor proteins. Moreover, an additional possibility has to be considered, namely that olfactory receptors on the antennae may function as contact gustatory receptors thus replacing the need for Grs (Robertson and Wanner 2006). Detecting the presence of gustatory molecular receptors is also not enough: quantitative PCR analyses and in situ hybridization studies should include all organs of the honeybee and not only those situated in the head. Functional tests aimed at characterizing the specificity of gustatory molecular receptors should be performed using a representative set of natural compounds. The choice of these compounds should take into account the different aspects of the taste biology of the honeybee. The world of taste of honeybees still has many mysteries to unravel. An ecological background combined with genomic, behavioral and electrophysiological analyses may guide such discoveries in a better way than just any of these approaches taken isolated.

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