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SHORT COMMUNICATION

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Highly controlled nest homeostasis of honey bees helps deactivate phenolics in nectar

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Abstract Honey bees have a highly developed nest homeostasis, for example, maintaining low CO_2 levels and stable nest temperatures at 35°C.We investigate the role of nest homeostasis in deactivating phenolic compounds present in the nectar of *Aloe littoralis*. We show that the phenolic content in nectar was reduced (from 0.65% to 0.49%) after nectar was incubated in a nest of *Apis cerana*, and that it was reduced still more (from 0.65% to 0.37%) if nectar was mixed with hypopharyngeal gland proteins (HGP) of worker bees before being placed inside a nest. HGP had little effect on samples outside a nest, indicating that nest conditions are necessary for HGP to deactivate phenolics in nectar. Consequently, the highly controlled nest homeostasis of honey bees facilitates direct deactivation of phenolics in nectar, and plays a role in the action of HGP as well.

Introduction

Secondary compounds are commonly present in nectar. More than 30% of the plant species in a survey by Baker (1977) (191 of 528 species) produced nectar containing phenolics and 10% of the plant species (50 of 567 species) secreted nectar with alkaloids. Nectar that contains secondary compounds, called "toxic nectar", frequently deters or poisons some floral visitors (Adler 2000), and honey bees, *Apis sp*., usually do not select toxic nectar as their food (see Adler 2000). Commonly, honey bees first process nectar into honey, instead of feeding on it directly (Winston 1987). Nectar sugars are externally inverted as a result of enzymes secreted by worker bees' hypopharyngeal glands; which express some carbohydrate-metabolizing enzymes for food processing. One of these enzymes is glucose oxidase (GOX) (Ohashi et al. 1999), which helps arthropods deactivate alkaloids (Musser et al. 2002).

As a result of this alkaloid deactivation, honey bees may be more resistant to alkaloid-containing nectar than adult Lepidoptera, and honey bees can exploit alkaloid-containing nectar as their food when few flowers are available (Baker 1977). Although GOX can break down glucose to release substantial amounts of H_2O_2, which then deactivates phenolics, phenolics can form irreversibly complexes with herbivores' enzymes when sufficient O_2 is present (Shi and Di 2000). This raises the possibility that GOX may be inhibited by phenolics in nectar outside the hives.

We hypothesized that homeostatic conditions in honey bee colonies, such as the maintenance of CO_2 levels and temperatures 35°C (Winston 1987), would create conditions under which phenolic compounds may be broken down, as well as prevent the formation of irreversible complexes of phenolics with proteins (Shi and Di 2000). Nest homeostasis of honey bees may play an important role in detoxification. We evaluated the role of nest homeostasis in deactivating phenolics in nectar.

Materials and methods

Preparation of HGP

Worker bees' hypopharyngeal gland proteins (HGP) was extracted using the method of Kubo et al. (1996). Foraging bees were collected at the entrance of their hives. The bees were anesthetized on ice, and their hypopharyngeal glands were dissected under a binocular microscope. The glands (100 glands/ml) were homogenized in buffered insect saline (containing 1 mM phenylmethylsulfonyl

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fluoride, 0.1 µg/ml pepstatin and 100 µg/ml leupeptin), and were centrifuged at 10000 rpm for 10 min. HGP in the supernatant was determined by the method of Bradford (1976), and diluted to 1 mg/ml by buffered insect saline.

Nectar sampling and treatments

The plant *Aloe littoralis* (Liliaceae) contains phenolics in its nectar; honey bees often avoid this nectar (Haglar and Buchmann 1993). Nectar was taken from *A. littoralis* at mid-morning of October 25, 2003, using a fine capillary tube, taking precautions to avoid any contamination by pollen. The phenolic content of the nectar was assayed for several treatments: (1) immediately after collection (Fresh); (2) incubated in the hive for 24 h (Hive); (3) exposed in the lab for 24 h mixed with HGP (Lab+HGP); (4) incubated in the hive for 24 h mixed with HGP (Hive+HGP); and (5) incubated in the hive for 24 h mixed with glutin (Hive+glutin) to evaluate the precipitation of phenolics by HGP in hives.

Each treatment had three replicates. For the treatments 2, 4, and 5 three samples of each treatment were bottled in vials with screened covers to prevent processing by worker bees. The vials were kept in separate colonies of *Apis cerana*, which maintained a mean of 1.80×10^{-2} CO_2 (by Qgd-07 CO_2 gasometer) at approximately 33.20°C (WMY-10 thermometer). For treatment 3, three samples were exposed to ambient air at 20.04°C in our laboratory.

Determination of phenolic content

1 ml (V) of each treated sample was mixed with 2 ml methanol (70%), and stored for up to 24 h in the lab. The supernatant was extracted, and diluted with sterile water to 50 ml (V_1). 0.5 ml (V_2) of the extraction was mixed with 1% $AlCl_3$ to 10 ml, and then centrifuged at 5000 rpm for 10 min. The absorbance (A) of the supernatant at 420 nm was measured. When the absorbance of the 1% $AlCl_3$ solution at 420 nm was 1.0, the phenolic content of the sample was $320AV_1/1000V_2V$.

Statistical analysis

The effects of the treatments on the phenolics contents in the nectar were compared using one-way analysis of variance (ANOVA) and multiple comparisons (LSD), with $\alpha = 0.05$.

Results

There was a significant treatment effect on the content of phenolics in nectar ($F_{4,10}$=9.871, p=0.002, Fig. 1). Fresh nectar of *A. littoralis* contained 0.6490±0.0825 mg/ml phenolics (mean±S.D., n=3). The phenolic content was reduced after nectar was incubated in a bee hive (0.4867±0.0132 mg/ml) (n=3) (comparing treatments 1 and 2, p=0.004). The breakdown of phenolics in the Hive + HGP treatment was 0.1623 mg/ml (=0.6490–0.4867).

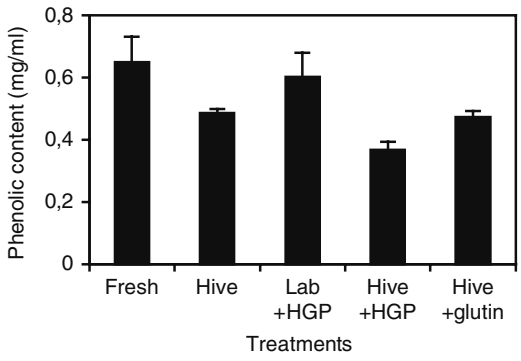

Fig. 1 Histogram of the phenolic content in nectar of *Aloe littoralis* for several treatments. Values are the means + S.D. See Materials and methods for explanation of the treatment names

HGP had little effect on the samples that were exposed in the lab (0.6027±0.0772 mg/ml) (n=3) (comparing treatments 1 and 3, p=0.309). Thus, GOX of HGP was inhibited outside the hives. When fresh nectar was mixed with HGP before being placed inside a hive, its phenolic content was significantly reduced (0.3667±0.0273 mg/ml) (n=3) (comparing treatments 1 and 4, $p<0.001$) (Fig. 1).

The large reduction of phenolics in nectar with HGP in the hive (0.6490–0.3667=0.2823 mg/ml) reflects the contribution of several factors, including the breakdown in the nest environment (0.1623 mg/ml, see above), the precipitation of phenolics by HGP, and the deactivation of phenolics by GOX. To evaluate the role of GOX in detoxification, we used glutin to evaluate the precipitation of phenolics by HGP in hives (treatment 5). The precipitation of phenolics by glutin was only 0.0127 mg/ml (n=3) (=0.4867–0.4740). Therefore, the deactivation of phenolics by GOX in hives was 0.1073 mg/ml (=0.2823–0.1623–0.0127).

Discussion

One of the advantages of insect social organization is colony homeostasis (Winston 1987). Highly controlled environmental conditions allow large changes from outside conditions. Here we have shown that the hive microenvironment can directly deactivate phenolics in the nectar, and that it plays a role in the action of HGP. Thus, the costs from consuming toxic nectar are reduced, and toxic nectar intake may require an increase in sugar supply. The detoxification of phenolics within bee colonies helps us explain the feeding performance of bees on toxic nectar.

Phenolics are produced for defense by plants. A high content of phenolics strongly deters many herbivores (Shi and Di 2000). Our results suggest that the content of phenolics could be considerably reduced after fresh nectar was inverted into honey. But worker bees prefer to collect the phenolics-containing nectar of *Prunus dulcis* than diluted

honey (Haglar and Buchmann 1993). Thus, worker bees prefer toxic nectar with a high content of sugar.

Nicotiana produces nectar with alkaloids (Detzel and Wink 1993). Worker bees refused to feed on *Nicotiana* flowers unless they were fed with syrup at the same time. Once the syrup was exhausted the bees stopped visiting *Nicotiana* as well (Faegri and van der Pijl 1979). Consequently, the need for sugar supply for detoxification within the hives may not be limited to deactivating phenolics, but might include deactivating a broad range of other toxins.

The feeding performance of bees may be a valuable topic in future investigations of the biology of toxic nectar. Toxic nectar is geographically widespread (Baker 1977), and is frequently present in plants that are interspersed with many other plants (Rhoades and Bergdahl 1981). When few other flowers are available, honey bees may intensively exploit toxic nectar as their food (Xu 1983). When exploiting toxic nectar, we expect the colony to simultaneously obtain sugar from non-toxic sources, thus promoting the worker bees to visit other sympatric plants. Other sympatric, synchronously flowering plants may benefit from the presence of plants with copious toxic nectar in terms of pollination.

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