

Amino acid content and nectar choice by forager honeybees (*Apis mellifera* L.)

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Abstract Dual choice feeding tests were performed to determine a preference of forager honeybees for specific amino acids. Artificial nectar containing proline was preferred over those containing only sugars. Nectar containing alanine was preferred on the first day, but preference was no longer significant thereafter. On the contrary, a negative response was found for serine. When the bees were given the choice between two nectars enriched with different compounds, proline was preferred above both alanine and serine, and alanine above serine.

Keywords Amino acid content · Feeding preference · Honeybee · Nectar

Introduction

Seed production is proportional to the efficiency of fertilization, thus the presence of pollinators can significantly increase crop yield (Allen-Wardell et al. 1998). Dicotyledonous species often attract insects by offering them a reward, primarily floral nectars. The nectar, an aqueous solution of sugars, amino acids, organic acids, proteins,

fats, vitamins, minerals and other minor components, is derived from the phloem sap and is produced by a group of specialized cells, called nectaries. Its composition can vary greatly depending on plant species and environmental conditions (Gardener and Gillman 2001a). Sugar content ranges 5–80%, and in most cases sucrose is the main component, whereas in others sucrose, glucose and fructose are present in similar amounts (Davis et al. 1998). Insects rely on nectar sugars for energetic expenditures, primarily flight. Amino acids are also found in the nectar but at much low quantities (typically 0.002–4.8% organic matter; Gardener and Gillman 2001b), and the biological significance of their presence is still being debated.

Plants pollinated by butterflies were shown to contain a higher concentration of amino acids in their nectar than species pollinated by birds (Baker and Baker 1986). The quantity and quality of these amino acids are believed to enhance insect longevity and fecundity (Mevi-Schutz and Erhardt 2005). Increasing evidence supports the preference of insects for sugar solutions enriched with amino acids (e.g. Rathman et al. 1990; Erhardt and Rusterholz 1998). This preference does not seem to exist in pollinator birds (Leseigneur et al. 2007). In nectars of numerous angiosperms, proline is the prevalent amino acid (Gardener and Gillman 2002; Kaczorowski et al. 2005; Carter et al. 2006; Terrab et al. 2007). Proline has been proposed to represent an energy substrate to fuel the earliest or most expensive stages of insect flight (Micheu et al. 2000; Gade and Auerswald 2002). A coevolution strategy has therefore been hypothesized by which increased proline content in the nectar, being an insect attractant, could increase plant visitation and thereby plant fitness.

Research on bees' preferences and the role of nectar amino acids has to date delivered inconsistent results. An early study (Inouye and Waller 1984) showed a general

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decline in nectar consumption for most amino acids as concentrations increased. A preference was found only for phenylalanine. In another study carried out with 37 different species, amino acid supplementation at 35–80 mM did not affect nectar attractiveness (Roubik et al. 1995). No preference effects were found in the case of a tropical bee (Gardener et al. 2003). More recently, a preference for proline-enriched artificial nectar was reported for honeybees (*Apis mellifera* L.), but no other amino acids were tested (Carter et al. 2006). So far, there has not been a conclusive study on general preferences for amino acid-containing fluids by bees. In our study we tested the preference of forager honeybees for proline, serine and alanine-enriched nectars, and report experimental data supporting an actual preference hierarchy between these amino acids.

Materials and methods

The research was carried out from July to September 2009. The apiary was located in Cadriano (Bologna, Italy) and consisted of nine healthy organic beehives, Dadant-Blatt type at 10 combs. Forager honeybees were caught at the entrance of the hives, briefly (20–30 s) narcotized with CO₂, and immediately distributed into disposable transparent plastic cages (14 cm D, 10 cm W, 4.5 cm H) with a 10.5 × 3.5 cm upper window covered with nylon mesh to allow gas exchange, 25 individuals in each cage. Cages were transferred into a thermostatic incubator at 33 ± 0.5°C in the dark, and honeybees were allowed to recover from the manipulation stress for 2 h with a sugar solution freely available from a feeder consisting of a 2.5 ml syringe whose nozzle had been cut. After recovery, the syringe was replaced with a feeder filled with distilled water, and two further syringes were inserted into the cage lid, in symmetrical positions and random order. These contained the two artificial test nectars. The sugar-only nectar consisted of 12% (w/v) glucose, 12% (w/v) fructose and 1% (w/v) sucrose, and had a density of 1.100 ± 0.002 g ml⁻¹. The amino acid nectar contained additionally either serine, alanine or proline and was added from a 1 M stock solution to a 10 mM final concentration. The addition did not influence the resulting pH, which ranged 5.6–5.9. All nutrient solutions were filter-sterilized (0.22 µm). After 24 h, feeders were removed, and the remaining artificial nectar was quantified by weight. Syringes were refilled with the corresponding sterile nectar, and the whole procedure was repeated every 24 h for up to 4 days. Each day the viable honeybees were counted, without removing dead insects, and the mean daily individual consumption of each nectar was calculated on the basis of the mean number of viable insects in a given cage during the previous 24 h period.

For every dual choice test, no less than 18 replications (cages) were run. Cages with less than eight honeybees remaining were excluded from the analysis. However, in all cases presented data are means ± SE of no less than 17 replications. The significance of differences in daily consumption between artificial nectars was analyzed by paired *t* test. For each experiment, a one-way ANOVA for repeated measures was performed to determine a possible effect of time on feeding preference. For all analyses the Statistica software package, Version 7.1 (StatSoft), was used.

Results and discussion

Within the frame of a project aimed at evaluating possible differences in nectar amino acid composition among genotypes of various crops and their consequence on honeybee attractiveness, the effect of various amino acid supplementations on the rate of artificial nectar consumption was investigated. In a first series of experiments, a dual choice feeding test was carried out under strictly controlled conditions in which forager honeybees were allowed to choose between an artificial nectar composed of glucose, fructose and sucrose similar to that found in natural rape-seed nectars (Davis et al. 1998), and an artificial nectar composed of the same sugars supplemented with either proline, alanine or serine. Amino acid concentration was set based on honeybees' preferences for proline (Carter et al. 2006), and concentrations close to those found in several natural nectars (Gardener and Gillman 2001b). Alanine and serine were selected because they are not related biosynthetically to proline, and belong to the same group of (neutral) amino acids. Results are summarized in Fig. 1. Bees showed a clear preference for proline-enriched nectar over nectar containing only sugars. Mean individual consumption of alanine-enriched nectar was also higher, but on the whole the difference was not statistically significant, and no effect of time on insect preference was evident ($F = 1.910$, $P = 0.140$). Interestingly, in the case of serine an opposite behavior was found, with control nectar preferred over that supplemented with the amino acid. This preference seems to increase with time ($F = 2.515$, $P = 0.069$). A disfavor for serine would imply a wider capability of insects to taste amino acids, which to date has been reported only in the case of proline and hydroxyproline (Hansen et al. 1998; Wacht et al. 2000).

A second series of experiments tested the honeybees' preference between artificial nectars containing different amino acids. Results (Fig. 2) fully confirmed the first set of data, with proline preferred over alanine, and both compounds preferred over serine. The difference between proline- (preferred) and serine-spiced (disfavored) nectars was indeed striking, and highly significant even if the

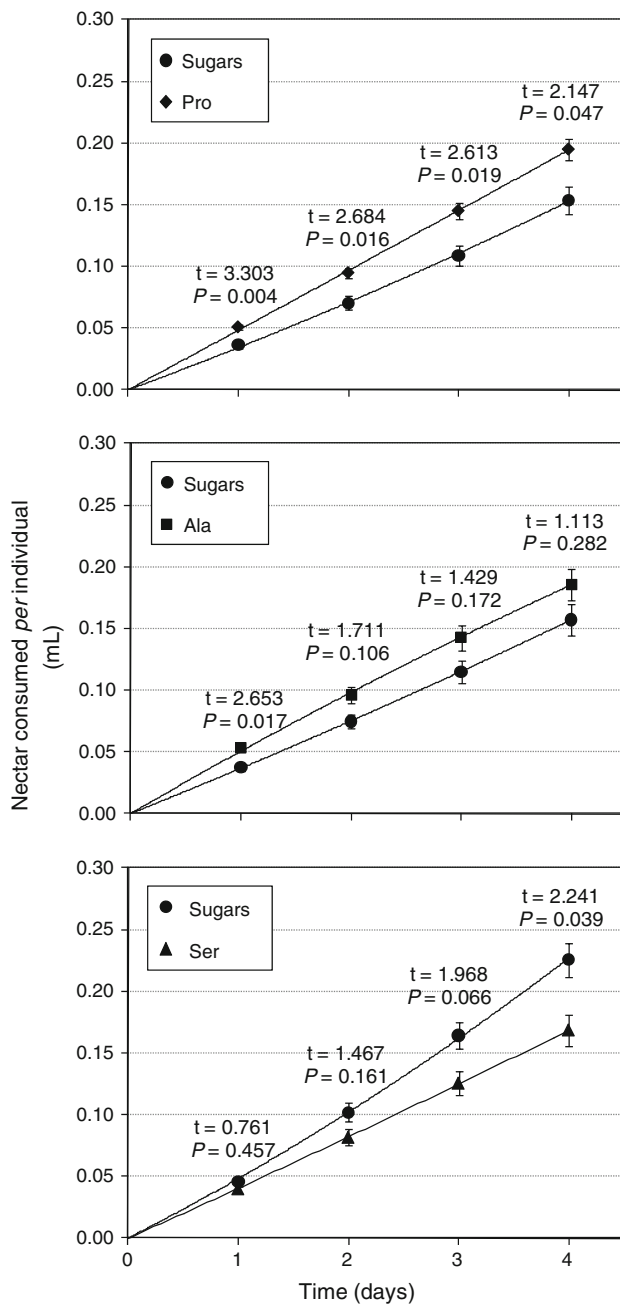


Fig. 1 Effect of amino acid supplementation on forager honeybee preference for artificial nectars. Bees were allowed to choose between an artificial nectar containing only sugars and the same nectar enriched with either 10 mM proline, alanine or serine. Mean individual consumption was measured daily. Results are means $\pm$ SE over at least 17 replications. Statistical significance of observed differences was determined by paired *t* test

Bonferroni correction for multiple *t* test is considered {significant at $P < [0.05/(n = 24) = 0.002]$ }. This is a surprising result, since serine was reported to be the main nectar amino acid in some plant species (Baker and Baker 1977). These preferences and aversions may influence the frequency of flower visitation by honeybees. Amino acid

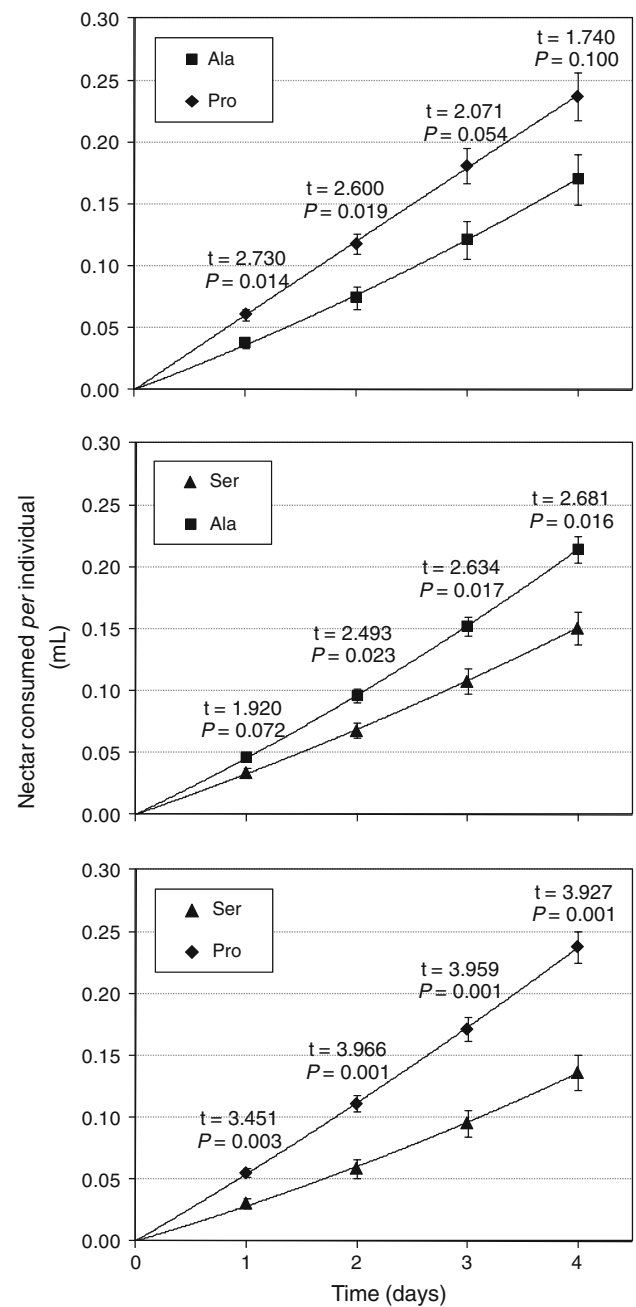


Fig. 2 Preference of forager honeybees for artificial nectars containing different amino acids. Bees were allowed to choose between artificial nectars containing sugars and either proline, alanine or serine, in all three possible combinations. Mean individual consumption was measured daily. Replications and statistical analysis as in Fig. 1

content in nectar could therefore contribute in providing the basis for flower constancy, the phenomenon by which an individual forager actually bypasses rewarding flowers to restrict visits to a single plant species (Sanderson and Wells 2005). It may represent as well a factor in plant–insect coevolution, since nonrandom patterns of phylogenetic congruence seem to rely at least in part upon tracking

of particular chemical traits (Percy et al. 2004). These results may also open new perspectives in both hive management and optimization of crop yield. The possible occurrence of a natural variability in nectar amino acid composition among cultivars of a bee-pollinated crop could in fact result in different seed set efficiency, significantly influencing final grain harvest. On the other hand, positioning hives near a field where a high-proline/low-serine nectar crop is cultivated could “force” the bees to visit this preferred source of nectar. Feeding on a single plant species, bees would produce a valued honey, with a distinctive aroma and flavor.

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