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The taste of nectar – a neglected area of pollination ecology

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Nectar is an important biological resource that is utilized by a wide range of animals as a food source. Amino acids are the second most abundant class of compound (after sugars) to be found in nectar. In foraging for nectar, animals carry out the vital role of pollination. Many animal taxa visit flowers, but the most abundant pollinators are insects. Although amino acids are detectable by insects, little work has focussed on the role of taste in the ecology of pollination (with most studies concentrating on foraging choice). The idea that different amino acids elicit different responses in insect taste receptors was used to characterize nectar samples from 65 plant species from a wide range of families according to their amino acid profile (determined by high performance liquid chromatography). A ternary classification system was used to map the amino acids present in nectar samples. There is a wide range of taste profiles with most plant species having their own characteristic taste value. How nectar tastes to pollinating insects is of great importance in understanding the foraging choices of insect pollinators and there are many avenues that remain to be explored.

Amino acids in nectar

Nectar is a key biological resource that is utilized by a wide variety of organisms as a food source. Insects are an especially important group of flower-visiting animals and the evolution of flowering plants (angiosperms) and insects, particularly bees (Hymenoptera) and butterflies (Lepidoptera) has often been linked (Burger 1981, Crane et al. 1995). Although the principal ingredient of nectar is sugars, typically in the range 15–40% w/v, it has long been recognized that nitrogenous substances are also present (Ziegler 1956, Lüttge 1961). However, not until the pioneering work of Baker and Baker (1973) was it realized that amino acids are ubiquitous in floral nectar, occurring at millimolar concentration. This discovery initiated a series of investigations into the composition and concentration of amino acids in nectar, and provoked debate concerning their ecological role as a resource (Baker and Baker 1975, 1982, 1983, 1986, Baker 1977, Gottsberger et al. 1984, 1989). To date, this debate has not been satisfactorily resolved but the consensus view is that plants that are adapted to pollination by butterflies show high concentrations of amino acids whilst plants pollinated by birds exhibit low concentrations of amino acids (Baker and Baker 1973, Baker 1982). The ecological rationale underlying this is that butterflies are specialized liquid feeders as adults and nectar is their only source of nitrogen (Hall and Willmott 2000). Birds, in contrast, also eat insects and so can gain nitrogen in the form of animal protein (Stiles 1971). Plants pollinated by bees, which are able to eat and digest pollen, form an intermediate group.

Responses of pollinators to amino acids

If we are to fully understand the role of amino acids in nectar, then the question of how pollinators respond to solutions containing them is of fundamental importance. Initial studies recognized that nectar amino acids might be detectable by insect visitors and may contribute to the overall taste of nectar (Baker and Baker 1977). Since that time a number of studies have examined this question using a variety of insect taxa to determine preference and foraging choice. Inouye and Waller (1984) found that honeybees, *Apis mellifera*, altered their feeding in response to various single amino acids whilst Alm et al. (1990) demonstrated honeybee preference for nectar mimic solutions containing a mixture of amino acids. A more recent study showed that glycine elicited a feeding response in honeybees (Kim and Smith 2000). Potter and Bertin (1988) found that the flesh fly *Sarcophaga bullata* preferred some amino acid-sugar mixtures to sugar-only controls but a later study found this preference only if animals were deprived of other protein sources (Rathman et al. 1990). This suggests that flies can detect amino acids and points to a role of nutrition in the feeding preferences of this taxa. The feeding preferences of various butterfly species have been examined with mixed results. In some

cases no preference was observed (Erhardt 1991, 1992, Romeis and Wackers 2000) whilst in others females showed a preference for nectar mimics with additional amino acids but males did not (Alm et al. 1990, Erhardt and Rusterholz 1998). More recent work on the adonis blue butterfly, *Lysandra bellargus*, showed that in the wild the sexes forage on different plant species with females choosing those containing a higher concentration of amino acids than males (Rusterholz and Erhardt 2000). These mixed results reflect the diversity of butterfly life history patterns but do point to a nutritive role for nectar amino acids in certain species. Although not generally pollinators, many ant species visit extrafloral nectaries and in many cases have a mutualistic defence relationship with the plants that provide nectar (Bentley 1976, Keeler 1977, Koptur 1984, Smiley 1985, 1986). Preference experiments suggest that some ant species can discriminate between sugar-only and sugar-amino acid mixtures and may exhibit a preference for the latter (Koptur 1979, Lanza and Krauss 1984, Lanza 1988, 1991, Lanza et al. 1993).

Chemoreception of amino acids by insects

Early work showed that insect chemoreceptors were capable of reacting to a wide range of amino acids in solution (Schoonhoven 1969). Subsequent work by Shiraishi and Kuwabra (1970) identified four classes of amino acid related to chemosensory response in two species of fly (flesh fly, *Boettcherisca peregrina* and blow fly, *Phormia regina*). The identities of these amino acids are summarized in Table 1. Class I amino acids elicited no response, class II inhibited the three types of chemosensory cell (salt, sugar and water), class III stimulated the salt receptor cell and class IV stimulated the sugar receptor cell. Dethier (1971) showed how even a small number of receptors (as few as 48 in a caterpillar compared to 10^8 for a rabbit) can respond to a wide range of stimuli to produce a sophisticated detection system. Many subsequent studies (Mullin et al. 1994) have examined chemoreception in insects but the study by Shiraishi and Kuwabra (1970) remains the only one to produce a systematic overview of chemoreception.

Table 1. Taste classes for various amino acids as described by Shiraishi and Kuwabra (1970) for two species of fly, *Boettcherisca peregrina* and *Phormia regina*.

Amino acid taste classes
Class I – no effect Asn, Gln, Ala, Cys, Gly, Ser, Thr, Tyr
Class II – general inhibitory Arg, Asp, Glu, His, Lys
Class III – salt cell stimulatory Hyp, Pro
Class IV – sugar cell stimulatory Ile, Leu, Met, Phe, Trp, Val

Current work

As a result of ongoing research (Gardener and Gillman 2001a, b), the amino acid composition of a number of plant species was available for examination. The current study aimed to examine the taste characteristics of nectar samples from a range of plant species and to stimulate further research into this neglected area of pollination ecology.

Methods

Identification and quantification of nectar amino acids

Nectar samples were collected using 5 µl glass capillaries. In total 446 samples were collected. These represented plants from a wide range of habitats including temperate grassland, mixed temperate woodland and tropical rainforest. The samples represented 65 species, 32 plant families and 16 orders (see Table 2 for list of species analyzed). High-performance liquid chromatography (HPLC) was used to determine the identity and concentration of amino acids in each sample in the following manner (detailed analysis of the composition of 30 species is presented in Gardener and Gillman (2001b), whilst compositional analysis of the others will be presented elsewhere).

Samples were derivatized using the AccQtag protocol (Waters Corp., Cohen and Micheaud 1993) in a 0.02 M borate buffer (pH 8.59). HPLC was performed, with standards every four samples, using the following equipment: Waters 712 WISP autosampler, Waters 600 pump controller, Waters 600 HPLC pump with 510 pump-heads. Separation was achieved using a Novapak C18 (15 cm × 4.6 mm) cartridge with guard column. The binary solvent system was a 6:4 acetonitrile/water mix and a TEA/phosphate (pH 5.04) buffer. Detection was via a Waters 474 scanning fluorescent detector (excitation at 250 nm and detection at 395 nm). The system was monitored and data collected using the Waters Millennium^{32} software. Chromatograms were analyzed and compared to standards for identification of individual amino acids. Standard amino acids were made up to a concentration of 100 pmol μl^{-1}. In addition to all the protein-building amino acids, standards of hydroxyproline, ornithine, taurine, AABA and GABA were used. Peak areas were compared to standards to determine the concentration of individual amino acids.

Table 2. List of species analyzed for nectar amino acid composition. Full compositional analyses are available from the authors.

Species	Family	Order
Thunbergia grandiflora	Acanthaceae	Lamiales
Catharanthus roseus	Apocynaceae	Gentianales
Thevetia thevetoides	Apocynaceae	Gentianales
Vinca major	Apocynaceae	Gentianales
Centaurea cyanus	Asteraceae	Asterales
Cirsium vulgare	Asteraceae	Asterales
Berberis thunbergii	Berberidaceae	Ranunculales
Jacaranda mimosefolia	Bignoniaceae	Lamiales
Pyrostegia venusta	Bignoniaceae	Lamiales
Tabebuia roseo-alba	Bignoniaceae	Lamiales
Borago officinalis	Boraginaceae	Euasterid
Nemophila Five Spot	Boraginaceae	Euasterid
Pulmonaria officinalis	Boraginaceae	Euasterid
Alliaria petiolata	Brassicaceae	Brassicales
Cardamine pratensis	Brassicaceae	Brassicales
Lunaria annua	Brassicaceae	Brassicales
Buddleja davidii	Buddlejaceae	Lamiales
Peltophorum pterocarpum	Caesilpinaceae	Fabales
Lonicera hecrotii Goldflame	Caprifoliaceae	Dipsacales
Lonicera periclymenum	Caprifoliaceae	Dipsacales
Agrostemma githago	Caryophyllaceae	Caryophyllales
Gypsophila elegans	Caryophyllaceae	Caryophyllales
Lychnis flos-cuculi	Caryophyllaceae	Caryophyllales
Silene dioica	Caryophyllaceae	Caryophyllales
Quisqualis indica	Combretaceae	Myrtales
Calystegia sylvatica	Convolvulaceae	Solanales
Convolvulus arvensis	Convolvulaceae	Solanales
Lathyrus odoratus Matucana	Fabaceae	Fabales
Lotus corniculatus	Fabaceae	Fabales
Pterocarpus indicus	Fabaceae	Fabales
Trifolium pratense	Fabaceae	Fabales
Vicia sativa	Fabaceae	Fabales
Corydalis lutea	Fumariaceae	Ranunculales
Ajuga reptans	Lamiaceae	Lamiales
Lamium album	Lamiaceae	Lamiales
Lamium purpureum	Lamiaceae	Lamiales
Prunella vulgaris	Lamiaceae	Lamiales
Salvia sp.	Lamiaceae	Lamiales
Stachys sylvatica	Lamiaceae	Lamiales
Barringtonia racemosa	Lecythidaceae	Ericales
Limnanthese douglasii	Limnanthaceae	Geraniales
Lythrum salicaria	Lythraceae	Myrtales
Lavatera arborea	Malvaceae	Malvales
Malva sylvestris	Malvaceae	Malvales
Melaleuca leucadendra	Myrtaceae	Myrtales
Chamerion angustifolium	Onagraceae	Myrtales
Epilobium hirsutum	Onagraceae	Myrtales
Epilobium montanum	Onagraceae	Myrtales
Ipomopsis aggregata	Polemoniaceae	Ericales
Primula veris	Primulaceae	Ericales
Primula vulgaris	Primulaceae	Ericales
Ixora coccinea	Rubiaceae	Gentianales
Aesculus hippocastanum	Sapindaceae	Sapindales
Aesculus x carnea	Sapindaceae	Sapindales
Scropularia scorodonia	Scrophulariaceae	Lamiales
Quassia amara	Simaroubaceae	Sapindales
Nicotiana Fragrant Cloud	Solanaceae	Solanales
Nolana paradoxa	Solanaceae	Solanales
Nasturtium Alaska Scarlet	Tropolaeolaceae	Geraniales
Nasturtium Cherry Rose Jewel	Tropolaeolaceae	Geraniales
Nasturtium Whirlybird Cherry	Tropolaeolaceae	Geraniales
Centranthus ruber	Valerianaceae	Dipsacales
Clerodendrum thomsoniae	Verbenaceae	Lamiales
Lantana montividensis	Verbenaceae	Lamiales
Lantana camara	Verbenaceae	Lamiales

Taste profile mapping

The existence of four distinct taste classes of amino acids (Fig. 1) permits the mapping of different mixes of amino acids to form a taste profile for any sample. Since taste class I amino acids are apparently undetectable (Shiraishi and Kuwabra 1970), the concentrations of taste classes II, III and IV were calculated for each nectar sample from the HPLC data. To visualize the data, ternary graphs were created where each axis represents the proportion a taste class contributes to the total. For each species the mean concentration of each taste class was calculated from replicate samples and these data were used to compare taste profiles between species (Fig. 1).

Taste groups of nectar

The results show that there is a wide range of taste profiles between species (Fig. 1). When samples for individual plant species are plotted, a clustered pattern is generally seen. For example, the nectar of honesty, *Lunaria annua* (Brassicaceae), shows a tendency for general inhibition of the chemoreceptors (triangles, Fig. 2). The nectar of field bindweed, *Convolvulus arvensis* (Convolvulaceae), shows a pattern that indicates stimulation of the sugar receptor (inverted triangles, Fig. 2). The nectar of selfheal, *Prunella vulgaris* (Lamiaceae), shows a tendency towards stimulation of the salt receptor (squares, Fig. 2), whilst purple loosetrife, *Lythrum salicaria* (Lythraceae), exhibits a more neutral cluster (circles, Fig. 2).

These findings suggest that plant species may have their own characteristic taste values. The amino acid composition (and so taste values) can be modified by soil nutrient conditions (Gardener and Gillman 2001a) by addition of pollen to nectar (Linskens and Schrauwen 1969, Erhardt and Baker 1990) or atmospheric CO_2 levels (Erhardt and Rusterholz 1997, Rusterholz and Erhardt 1998).

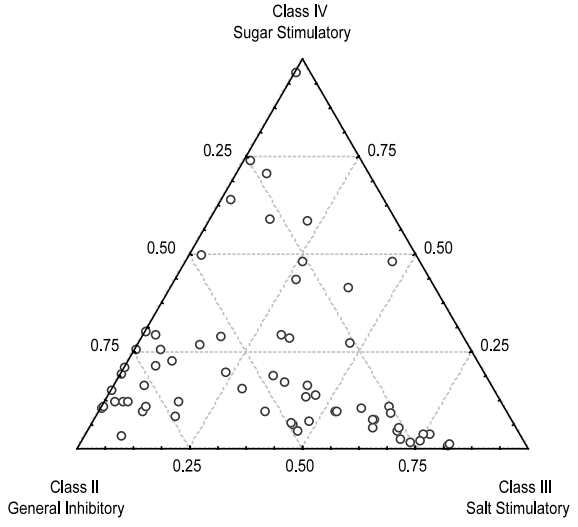

Fig. 1. Amino acid taste profiles from nectar for 65 plant species. Each axis represents the relative abundance of a taste class. Class II = general inhibition, class III = salt cell stimulation, class IV = sugar cell stimulation.

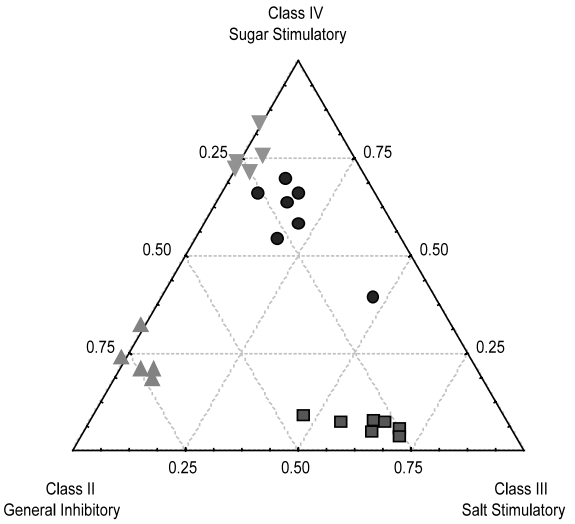

Fig. 2. Amino acid taste profiles for four plant species. Each axis represents the relative abundance of a taste class. Class II = general inhibition, class III = salt cell stimulation, class IV = sugar cell stimulation. ▲ = *Lunaria annua*, ▼ = *Convolvulus arvensis*, ● = *Lythrum salicaria*, ■ = *Prunella vulgaris*.

Discussion

The taste profiles generated here represent the taste as likely to be perceived by fly species, as the original study was conducted on this taxon (flesh fly, *Boettcherisca peregrina* and blow fly, *Phormia regina*) (Shiraishi and Kuwabra 1970). However, since no comparable data exist on the responses of other insect taxa, this method of representation is the best available at this time. Furthermore, the taste profiles were generated using relative abundance of the taste classes. The concentration of amino acids varies enormously between species (Baker and Baker 1973) and can also be highly variable within a single species (Gardener and Gillman 2001b). It is therefore possible that two species (or individuals of the same species) may have widely different concentrations but share the same apparent taste profile. This is not an insoluble problem as it is possible to present the data in other ways, e.g. a 3-dimensional graph. Alternatively, the balance between the three taste classes may be regarded as contributing most to the flavour (as perceived by the animal) whilst the overall concentration of the compounds informs as to the strength of that particular flavour. In other words,

the animal perceives two solutions with the same taste profile but different concentrations as being two strengths of the same flavour.

The taste of nectar to floral visitors must be of great importance. For instance, addition of pollen releases amino acids into nectar (Linskens and Schrauwen 1969, Erhardt and Baker 1990). The shift in the taste of nectar could form the mechanism by which a visiting insect could detect that the nectar was enhanced with extra amino acids. This may be of vital importance to the survival and fecundity of the insect (Gilbert 1972). Females require more resources for oogenesis than males do for spermatogenesis, whilst in some species transfer of nutrients at mating may be important (Boggs and Gilbert 1979). For other species, adult feeding on nectar amino acids may have a greater role in gametogenesis (first postulated by Watt et al. 1974). The differential foraging behaviour of male and female adonis blue butterflies shown by Rusterholz and Erhardt (2000) points to a role for nectar amino acids in reproduction in this species; the pattern may be more widespread.

Amino acids are by no means the only compounds in nectar to contribute towards the taste. Sugars are by far the most abundant compound in nectar and must surely dominate the taste of nectar. Many experiments have demonstrated animal responses to different sugars (Ricks and Vinson 1970, Stiles 1976, Erhardt 1992, Bartareau 1996, Roberts 1996). How far nectar amino acids contribute to the overall taste of nectar is largely unknown. There are also other compounds present that may affect the taste (e.g. lipids, phenolics, Baker and Baker 1982) but their contribution is, once again, unknown. This demonstrates that analysis of the taste of nectar is a subject that has a great deal of research potential.

Future work

The relative importance of the taste of amino acids compared with sugar needs to be determined, especially for adult animals that cannot obtain amino acids by means other than nectar (e.g. most butterflies and moths). The model utilized here was generated from studies on fly species. Further work needs to be conducted to determine how the taste receptors of different insects react to amino acids and to refine the taste model by examining the relative strengths of response generated by each amino acid. The taste perceptions of male and females from the same species may prove interesting in the light of studies that have shown differences in food choice. This is of fundamental importance if we are to fully understand the ecological role of amino acids and taste in the foraging ecology of flower-visiting animals.

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