Plant Syst Evol (2009) 283:133–147
DOI 10.1007/s00606-009-0215-3

ORIGINAL ARTICLE

Composition of the floral nectar of different subgenera of Argentinian *Passiflora* species

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Received: 27 February 2009 / Accepted: 20 August 2009 / Published online: 10 September 2009

Abstract The composition of the floral nectar sugars and amino acids of four species of *Passiflora* (*P. foetida*, *P. caerulea*, *P. suberosa*, and *P. misera*) included in different infrageneric taxa and with distinct pollination mechanisms has been studied. The effect of weather and floral age on nectar volume, existence, and total and relative amounts of the various compounds was explored. The proportion of sugars was rather constant within a given species whereas the composition, number, and total quantity of amino acids showed great intraspecific and intraplant variability; these nectar properties were independent of floral stage or meteorological conditions. Species belonging to the same subgenus displayed equivalent sugar ratios and similar total amount of amino acids, so these characteristics might be conservative in the genus. For all species, the amino acid concentration surpassed known values for their respective pollination syndromes, viz. bee and wasp-pollinated flowers. No relationship emerged between pollinators with different glossa length and nectars with distinct sugar ratios. Rather, nectar chemical composition seems to reflect taxonomic relationships.

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Keywords Floral nectar constituents · Sugars · Amino acids · *Passiflora* · Pollination mechanisms · Chemotaxonomical affinities

Introduction

Studies dealing with nectar constituents are numerous (Wykes 1952; Handel et al. 1972; Baker and Baker 1973, 1975; Käpilä 1978; Southwick et al. 1981; Baker and Baker 1983a, b; Freeman and Worthington 1985; Baker and Baker 1986; Bernardello et al. 1994; Nicolson 2007). Temporal fluctuations in secretion and concentration, in relation to either meteorological conditions or time of day, have been studied almost exclusively for sugars (Corbet 1978; Plowright 1981; Southwick et al. 1981; Cruden et al. 1983; Pleasants 1983; Plowright 1985; Wyatt et al. 1992). Correlations between the proportions of sugars (Wist and Davis 2006) or amino acid composition (Gottsberger et al. 1990; Petanidou 2005) and floral age, or between amino acids and weather conditions (Baker and Baker 1977 cited in Nicolson and Thornburg 2007) have rarely been performed.

Although Baker and Baker (1983a) found some correlations between pollinators and nectar chemistry (sugar ratios, amino acid amounts), they emphasized that phylogenetic constraints may be acting when those correlations do not appear. These authors realized the conservatism in the proportion of the three main sugars (sucrose, fructose, glucose) within certain families but also the marked differences even between closely related species (Baker and Baker 1983b). Some correlation between nectar sugar composition and sugar dominance with pollination syndromes in a tribe of Gesneriaceae was encountered by Perret et al. (2001), and between sugar ratio and pollinators

in hummingbird-pollinated species by Bernardello et al. (2000), but no relationship between nectar chemistry and pollinator type was found either by Galetto et al. (1998) or Galetto and Bernardello (2004). Similar nectar sugar composition and proportions correspond to taxonomic positions rather than to pollination characteristics in several tribes of Papilionoideae (van Wyk 1993) and in 35 species from nine tribes of Asteraceae (Torres and Galetto 2002). Distinct groups according to nectar sugar composition and proportions could be assigned to species included in the same sections of *Rhododendron* (Harborne 1985), in the same tribe (Galetto 1995), or at suprageneric level within a subfamily of Asphodelaceae (van Wyk et al. 1993), and even in different subspecies (Freeman et al. 1985), yet Witt et al. (1999) and Perret et al. (2001) indicated that few studies compared nectar composition and taxonomic relationship and emphasized the need to cover this aspect better.

The pattern of nectar secretion and lipid and mineral composition of the nectar of four *Passiflora* species studied by Varassin et al. (2001) were strongly associated with pollinator type. In *Passiflora*, some floral nectar characteristics have been examined. The sugar quality of *P. subpeltata*, *P. quadrangularis*, *P. mollissima*, *P. gracilis*, *P. caerulea*, and *P. antioquensis* was studied by Percival (1961), but neither the total amount of sugar nor the exact amount of each sugar was measured. Corbet and Willmer (1980) analysed the amount of glucose and total amino acids in *P. edulis*. Durkee et al. (1981) reported the presence and relative proportions of sugars and total amount of amino acids in *P. warmingii*, *P. biflora*, and *P. trinifolia*, but not the qualitative composition of the amino acids. Varassin et al. (2001) estimated glucose concentration by colorimetric analysis, but not the other main sugars, in species pollinated by different agents (*P. alata*, *P. speciosa*, *P. galbana*, and *P. mucronata*). There are also reports available on the amount of nectar of *P. caerulea* (Fahn 1979) and of sugars and amino acids of *P. alata* (Gottsberger et al. 1984).

The flowers of *P. foetida* L., *P. suberosa* L., *P. caerulea* L., and *P. misera* Kunth vary in size, time of anthesis, colour, odour, degree of self-compatibility, and pollinator taxa (Amela García 1999). The only aspects of nectar studied for these species are secretion pattern, reposition, and concentration (Amela García 1999). The exact composition of the floral nectar of these taxa has not yet been studied. The objective of this work was to compare the nectar constituents of these four species of *Passiflora*, which belong to different infrageneric categories and have different pollination mechanisms. The hypotheses postulated were that species with different pollination mechanisms and with different taxonomic positions might have different nectar chemistry.

Materials and methods

Species and study sites

Passiflora foetida, *P. caerulea*, *P. misera*, and *P. suberosa* occur from northern South America to Argentina (Killip 1938). In Argentina, they are distributed from the north to the centre (Deginani 2001). The study plants *P. foetida*, *P. misera*, and *P. suberosa* were obtained from seeds produced by specimens collected in their habitats of origin: Charata (Chaco province), Concordia (Entre Ríos province), and San Ignacio (Misiones province), respectively; they were grown in a partially-open greenhouse of the Faculty of Exact and Natural Sciences of Buenos Aires University (*P. foetida*, *P. misera*, and *P. suberosa*) and in a garden in Merlo, Buenos Aires province (*P. misera*), where *P. caerulea* individuals occurred naturally.

Floral biology and pollinators

Flowers of *Passiflora* species are solitary and bear a single annular nectary at the base of the corona. The nectar is concealed in a chamber closed by an operculum. The flowers display three floral stages (Amela García 1999); the first and the third are staminate stages and the second is staminate and pistillate. These stages were defined on the basis of the style movements. Nectar is produced in all stages (Amela García 1999). *P. caerulea* is mainly pollinated by carpenter bees (Amela García and Hoc 1997), *P. foetida* and *P. misera* by medium-sized bees (Gottsberger et al. 1988; Amela García 1999) and *P. suberosa* by bees and wasps (Koschnitzke and Sazima 1997).

Nectar sampling and analysis

Samples were taken from April 2003 to December 2005. Throughout manipulation of the samples nitrile gloves were used to prevent contamination. The flowers were emasculated the day before anthesis, before anther dehiscence, to prevent contamination with pollen when collecting nectar, because pollen increases amino acid content (Gottsberger et al. 1990). They were then bagged until nectar collection, to exclude visitors. Nectar was collected during each floral stage. Each flower was sampled once and the floral stage was recorded. To obtain the total volume in each stage, nectar of each flower was depleted in each sampling session. Thus, the secretion pattern could be evaluated. For some samples the following weather conditions were recorded at the time of sampling: shade or direct sun over the plants, sunny versus cloudy, rain, windy versus calm, relative humidity, and ambient temperature; the last two were recorded with a digital thermohygrometer, placed 2 m above ground. Nectar was extracted with

sterile calibrated end-to-end microcapillary tubes (Hirschmann Laborgeräte, Germany) and the volume recorded. Nectar was fixed in Eppendorf vials previously filled with 1 ml ethanol (70%). The nectar from the capillary was dispensed into the vial by means of a syringe applied to the capillary by a flexible plastic tube. Fixations were maintained in a freezer until HPLC analysis at Ulm University.

The liquid content of the nectar samples had to be replaced by a known volume of water for the HPLC analyses. Therefore, the samples were vacuum-centrifuged at 65°C (SpeedVac SC 110, Savant Instruments, USA) until all liquidity vanished and only the nectar's solid components were left. These components were dissolved in 100 µl water (HPLC-grade) and purified by filtering (0.45 µm pore size; Acrodisc syringe filter with Nylon membrane, 4 mm, Pall Corporation, USA).

Sugars

The following HPLC components were used: autosampler 717 plus, pump 510, refractive index detector 410, and a column heater module (all Waters, USA). Isocratic separation was achieved using a 72:28 acetonitrile–water mix (Schmidt 1998) with a flow rate of 1.4 ml min^{-1} and a high-performance carbohydrate column (4.6 × 250 mm; Waters) with a guard column. Operating temperature was 35°C. The mobile phase was degassed with helium 4.6 at a flow rate of 20 ml min^{-1}. The system was monitored and data collected by use of Millennium32 3.0.5 software (Waters). External standards were run for sucrose, fructose, and glucose (Merck, Germany).

Amino acids

Amino acid analysis followed the Millipore (1993) AccQ·Tag method. Samples (5 µl) were derivatized with AQC (6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate) in borate buffer (without previous hydrolysis). HPLC was performed using the following equipment: autosampler 717plus, 600E system controller, 470 scanning fluorescence detector, sat/in module, and a column heater module (all Waters). Separation was achieved using an AccQ·Tag column (Novapak C18, 3.9 × 150 mm, Waters) with a guard column. The gradient profile by Schmidt (1998) was used for the three eluents (a: sodium acetate, triethylamine (TEA) buffered with phosphoric acid (pH 5.5), ethylenediamine-tetraacetic acid (EDTA); b: acetonitrile; c: water) with a flow rate of 1 ml min^{-1} at an operating temperature of 37°C. The mobile phase was degassed with helium 4.6 at a flow rate of 20 ml min^{-1}. The system was monitored and data were collected by use of Millennium32 3.0.5 software. External standards were run for alanine, arginine, asparagine, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine (amino acid hydrolysate standard, Waters Corporation, USA).

Data analysis

All the variables were submitted to ANOVA with the InfoStat program. Statistical analysis was performed with the amount of each sugar; percentages are shown for comparative purposes. The total amount of sugars was expressed per volume unit and per flower, to enable comparison with different bibliographic sources.

Results

Nectar volume

Nectar was present from flower opening onward, and was secreted throughout anthesis (Fig. 1). Equivalent (*P. caerulea*) or more (the other species) volume was found in stage 2 compared with stage 1; minor (*P. foetida*), equivalent (*P. misera*), or more (*P. caerulea* and *P. suberosa*) volume was secreted during stage 3. The estimated total volume (µl) secreted during the flower's lifespan averaged 33.37 ($n = 25$), 169.75 ($n = 7$), 29.31 ($n = 17$), and 4.91 ($n = 9$) for *P. foetida*, *P. caerulea*, *P. misera*, and *P. suberosa*, respectively.

Sugars

Fructose, glucose, and sucrose were present in *P. foetida* and *P. caerulea* but usually only fructose and sucrose in *P. misera* and *P. suberosa* (Table 1).

The total amount of sugars in *P. foetida* was almost half that in the other species (Table 1); the greatest total amount of sugars was found in *P. suberosa*. The total amount of sugars (Table 1) in *P. foetida* differed significantly from that in the rest of the species ($F = 41.02$, $P < 0.0001$) and the amount of each sugar differed between all of them, except between *P. misera* and *P. suberosa* (fructose: $F = 113.21$, $P < 0.0001$; glucose: $F = 127.53$, $P < 0.0001$; sucrose: $F = 183.00$, $P < 0.0001$). The quantity of either total sugar or each sugar is rather constant within a given species, as is reflected by the small values of standard deviation displayed (Table 1).

With regard to the predominance of sucrose versus hexoses, *P. foetida* and *P. caerulea* are hexose-rich whereas in *P. misera* and *P. suberosa* sucrose is predominant. The total amount of sugars was similar between the different floral stages in each species (Fig. 2). The relative proportion of sugars in the four species kept constant during the three floral stages (Fig. 3).

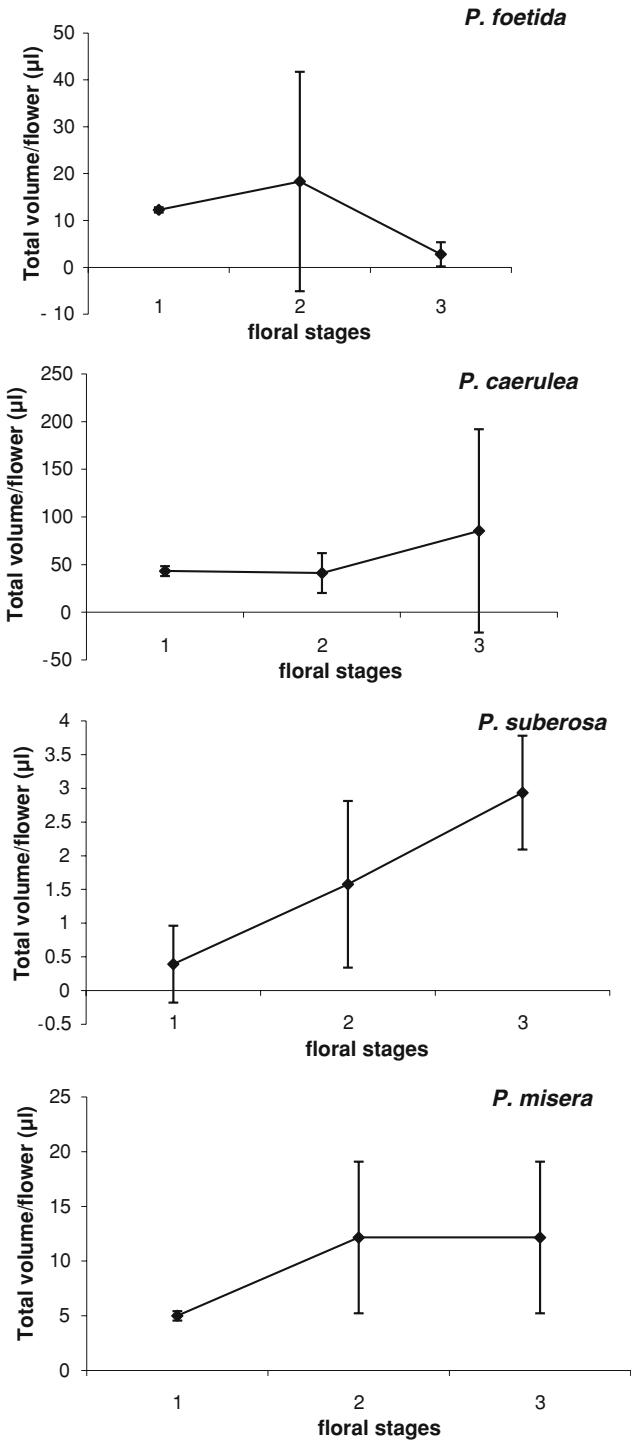

Fig. 1 Total amount of floral nectar at the three floral stages

The presence of each sugar in each floral stage varied between samples (Table 2), except in *P. caerulea*. In *P. foetida*, fructose and glucose were always present, whereas sucrose appeared in most of the samples in stage 3. In *P. misera*, fructose and sucrose were always present whereas glucose appeared in seven of the 17 samples in phases 1, 2, and 3. In *P. suberosa*, sucrose was present in all samples and glucose was absent from all samples, whereas fructose appeared in eight of 13 samples in the three phases.

There was no clear correlation between the total volume of nectar per flower and temperature or humidity (Figs. 4, 5). As ambient temperature increased and relative humidity decreased, there was a slight tendency for the total amount of sugars to increase (Figs. 6, 7). In *P. foetida*, for which on two sampling occasions it was rainy (vs. sunny on the remaining two), sugar production was nearly half (Figs. 6, 7, points with 99% and 19°C, respectively). On most of the sampling sessions for the other species were sunny; exceptions were two occasions for *P. suberosa*, when it was cloudy, but no clear tendency was perceived. Wind did not seem to affect sugar production as it blew on 2/3 of the sampling occasions for *P. misera* but all the samples were rather constant.

The sugar ratio did not vary with ambient temperature or relative humidity, nor with rain, irradiation (direct sun or shade over the plants), or air movement (calm vs. wind) (Table 3), as evidenced by results from samples taken under different meteorological conditions (*P. foetida* and *P. misera*).

Amino acids

The composition, number, and quantity of amino acids were quite variable between flowers of the same species, even from the same plant (Tables 4, 5). Not always the same amino acids appeared in all samples of each species (Table 4). Cysteine did not appear in any sample in any species and glycine, tyrosine, methionine, and phenylalanine were the least frequent. The amino acids that appeared in all the samples (i.e., in all the floral stages) were serine, glutamic acid, histidine, valine and lysine (in *P. misera*), arginine and threonine (in *P. misera* and *P. caerulea*), and alanine and proline in all species. Comparing the presence of amino acids along the lifetime of the flowers, tyrosine, lysine, leucine, and phenylalanine varied in stages 2 and 3 of *P. foetida* whereas asparagine, serine, glutamic acid, glycine, histidine, arginine, threonine, valine, and isoleucine varied in stage 3 in this species; stage 1 was the most consistent for these compounds in *P. caerulea*; the appearance of asparagine, serine, valine, lysine, and isoleucine varied within each floral stage in *P. suberosa*, whereas the appearance of glutamic acid, glycine, histidine, arginine, threonine, and leucine varied in only one of the floral stages and phenylalanine was absent from all samples; asparagine, isoleucine, leucine, methionine, and phenylalanine were present in different floral stages in *P. misera*. With regard to the amount of each amino acid, proline had the highest concentration followed by alanine, histidine, arginine, and threonine in all the species, and also glutamic acid and serine in *P. misera*.

Table 1 Total amount, composition, and quantity of sugars in the floral nectar of *P. foetida*, *P. caerulea*, *P. misera*, and *P. suberosa*

Species	*P. foetida*	*P. caerulea*	*P. misera*	*P. suberosa*
Total amount of sugars (μg/μl) $X \pm$ SD	237.04 ± 96.74^{a}	512.00 ± 49.89^{b}	513.00 ± 71.48^{b}	543.49 ± 147.08^{b}
Total amount of sugars (mg/flower)	1.28 ± 0.52	$24.\ 54 \pm 2.39$	5.02 ± 0.70	0.98 ± 0.26
Fructose (%)	44.41 ± 0.05^{b}	35.51 ± 0.03^{c}	2.15 ± 0.00^{a}	1.54 ± 0.01^{a}
Glucose (%)	44.11 ± 0.05^{b}	36.80 ± 0.03^{c}	0.25 ± 0.00^{a}	0 ± 0^{a}
Sucrose (%)	11.47 ± 0.09^{b}	27.69 ± 0.06^{c}	97.60 ± 0.01^{a}	98.45 ± 0.01^{a}
Sucrose/hexoses ratio	0.13	0.38	40.68	63.61
Flowers (*n*)	25	7	17	13
Plants (*n*)	12	2	3	6

Values with different letters differ significantly from each other

The total amount of amino acids was similar for *P. foetida* and *P. caerulea*, and less than for *P. misera* and *P. suberosa* (Table 5). The total amount of amino acids did not vary in a similar way along the floral stages between the species (Table 5): it was maximum in stage 3 for *P. foetida*, in stage 1 for *P. caerulea*, and in stage 2 for *P. misera* and *P. suberosa*. Despite the great intraspecific variability, the total amount of amino acids (Table 5) did not differ significantly between species ($F = 2.85$, $P > 0.05$). This result did not vary with temperature or humidity (Table 6); even sample 6, taken on a different day from sample 1 but under equivalent weather conditions, was three times more concentrated.

Discussion

Nectar volume, anthesis, sugar concentration, and pollinators

All the species investigated had nectar as soon as the flowers opened. Durkee et al. (1981) observed that the nectar was copious when the flowers of *Passiflora warmingii*, *P. biflora*, and *P. trinifolia* began anthesis, but they did not clarify what happened afterwards, nor the pollination syndromes. Bee and hummingbird-pollinated flowers secrete small quantities of nectar throughout anthesis and thus ensure visits during long periods; in contrast, secretion of nectar by bat-pollinated species is before anthesis, ensuring the large amount required by their pollinators (Varassin et al. 2001). The secretion pattern of *P. caerulea* was different from that obtained by Amela García and Hoc (1997), in which the greater volume appeared in floral stage 2, as for *P. foetida* (both in that study and this). Nevertheless, other species (*P. mooreana*, *P. chrysophylla*) also show increasing volume with increasing flower age (Amela García 1999). *P. misera* is the only one of all the analysed species in which volumes in stages 2 and 3 were equal (Amela García 1999 and this work). Nectar secretion frequently exhibits a daily peak (Búrquez and Corbet 1991), which would be attractive to visitors, increasing the probability of pollen removal and pollen transfer. All *Passiflora* species have three floral stages: in all the species, stage 2 is the only one in which both pollen deposition and removal can be performed, so the greater volume would be expected then. The degree of variability in nectar volume has been attributed to pollinator foraging behaviour: species with different pollination mechanisms differed in the moment of anthesis at which the variability of nectar volume was greater (Varassin et al. 2001). Nectar volumes are notorious for their variability, both among and within plants of the same species (Kearns and Inouye 1993; Nepi et al. 2003), and even between separate nectaries of the same flower (Herrera et al. 2006). This variability may reflect different photosynthetic activity, water availability, or exposure to varying meteorological conditions (Pacini et al. 2003). *P. foetida* total volume per floral stage was nearly a factor of six less than that obtained for the same species (Amela García 1999); the volumes for the other species were similar in both studies.

The estimated total volume secreted during the flower's lifespan was greatest in *P. caerulea*, less in *P. foetida*, even less in *P misera*, and lowest in *P. suberosa*. This is correlated with flower size (*P. caerulea* > *P. foetida* > *P. misera* > *P. suberosa*; measured by Amela García 1999), and, consequently, with nectary size, but not with anthesis, because that of *P. foetida* is the shortest.

Both the amount and volume of sugar present in *P. suberosa* and *P. foetida* fall in the ranges for bee-pollinated species (Cruden et al. 1983); on the other hand, the values for *P. caerulea* and *P. misera* fit more the quantities of sugar sucked by bats and hawkmoths, and by sunbirds, orioles, hawkmoths, or hummingbirds, respectively. This could be explained for *P. caerulea* by assuming that large bee-pollinated flowers could be derived from those pollinated by hummingbirds (Grant and Grant 1968, cited in Baker and Baker 1983b), and this is supported by the fact that hummingbirds visit this large passion flower (Amela García and Hoc 1997) and that an ornithophilous syndrome exists in

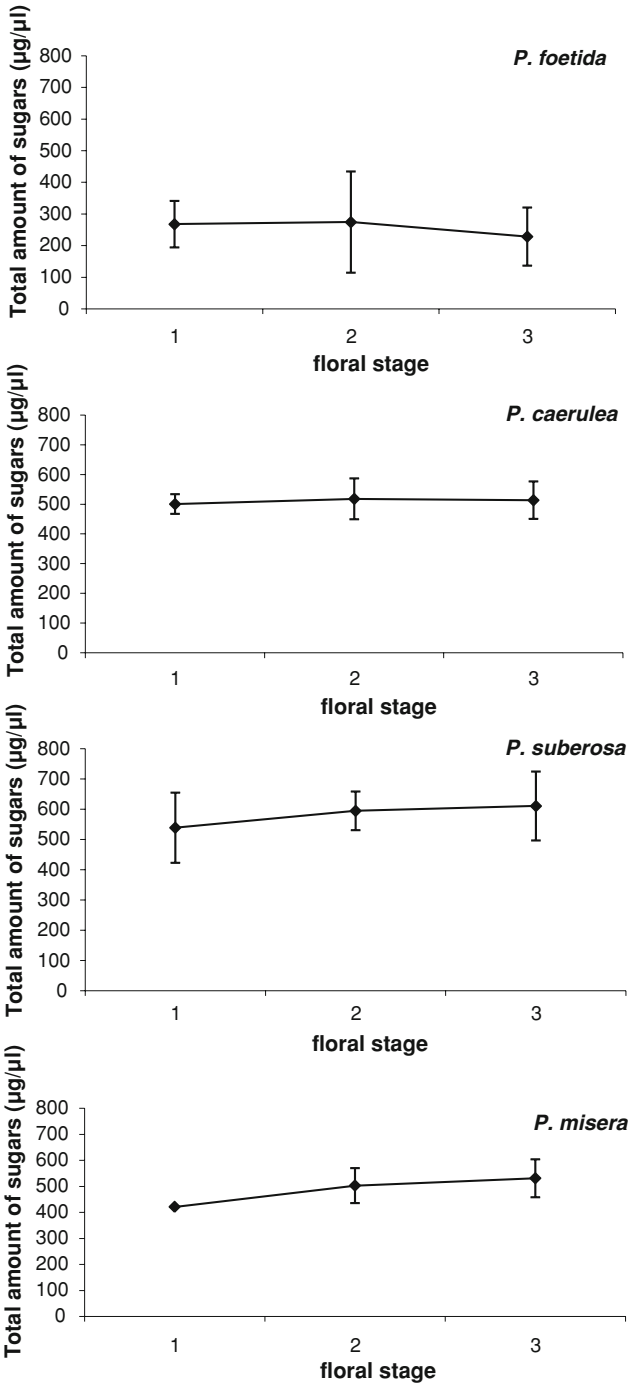

Fig. 2 Total amount of sugar in the three floral stages

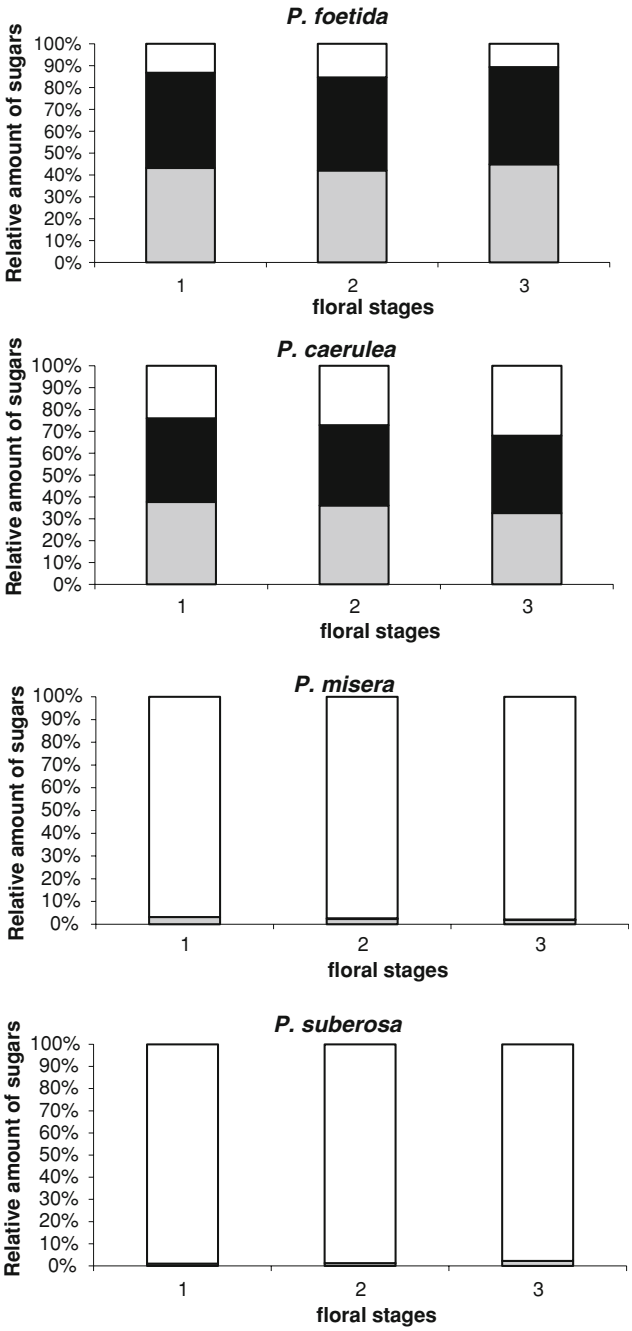

Fig. 3 Relative amount of sugars in the three floral stages; *black* = sucrose, *white* = glucose, *grey* = fructose

other *Passiflora* species (Vogel 1990). Total amount of sugar in *P. foetida* fits the value for Old World birds whereas that for *P. caerulea*, *P. misera*, and *P. suberosa* falls in the range for short-tongued bees (Gottsberger et al. 1984).

Sugar amount

The greater total amount of sugars in *P. suberosa* (mainly wasp-pollinated), in contrast with the other species investigated (bee-pollinated), may be related to pollinator type; the scanty data on the preferences of wasps account for a 30% sugar concentration (Spurr 1996). If anthesis is considered, the smaller total amount of sugars (nearly half) in *P. foetida* compared with the other species analysed, might be because of the shorter anthesis of *P. foetida* (dawn to late morning compared with dawn to sunset for *P. misera* and *P. suberosa* and morning to night for *P. caerulea*) (Amela García 1999). The maintenance or

Table 2 Presence of the different sugars according to floral stage

Species	Stage 1	Stage 2	Stage 3
P. foetida	f, g, s: 1 (1)	f, g, s: 1 (1)	f, g: 5 (5)
$n = 25$			f, g, s: 18 (10)
P. caerulea	f, g, s: 2 (2)	f, g, s: 3 (3)	f, g, s: 2 (2)
$n = 7$			
P. misera	f, s: 1 (1)	f, s: 3 (1)	f, s: 6 (3)
$n = 17$	f, g, s: 1 (1)	f, g, s: 3 (1)	f, g, s: 3 (3)
P. suberosa	f, s: 1 (2)	f, s: 2 (2)	f, s: 5 (3)
$n = 13$	s: 2 (2)	s: 2 (2)	s: 1 (1)

The table gives number of samples in which each sugar occurred. The numbers in parentheses are the numbers of plants

n is the total number of samples analysed

f, fructose, g glucose, s sucrose

small increase in the amount of sugars through anthesis in *P. misera* and *P. suberosa* suggests constant secretion, as was also found for *P. edulis* (Corbet and Willmer 1980). In contrast, the decrease in the amount of sugars during floral stages 2 and 3 for *P. foetida* and *P. caerulea*, although slight, together with the decrease in volume during stage 3 for *P. foetida* (cf. Figs. 1 vs. 2), could be because of resorption. Varassin et al. (2001) also detected sugar amount decrease in two species of *Passiflora* among the four examined, and interpreted it as resorption.

Sugar dominance

Nectar in five of the six *Passiflora* species examined by Percival (1961) was sucrose-dominant and one was hexose-dominant. This author considered the passion flowers as long-tubed; although not all species analysed by her have a deep hypanthium. In all passion vines the nectar is concealed, because of the morphology of the corona in which access to the nectar must be opened by the suitable visitor. Moreover, even the species with shallower nectar chambers and weaker opercula, *P. misera* and *P. suberosa* (Amela García 1999), had sucrose-dominant sugars.

Percival (1961) stated that the sugar proportions of *P. caerulea* varied among samples, from sucrose-dominant to fructose–glucose-dominant, based on four samples from a single plant analysed by paper chromatography, a method not as accurate as HPLC. The presence of the different sugars was quite constant in the three stages of the species studied here. The major variation occurred in *P. foetida* in stage 3, in which five out of 23 samples exhibited only fructose and glucose; this could be because of degradation of the sucrose in these hexoses in this last floral stage. The proportion of sugars was independent of the floral age or weather for all the species. The maintenance of the sugars' relative proportions during anthesis within a species is in accordance with most data in the literature (Wykes 1952;Baker and Baker 1983a; Harborne 1985; Nicolson and Thornburg 2007); nevertheless, changes in nectar

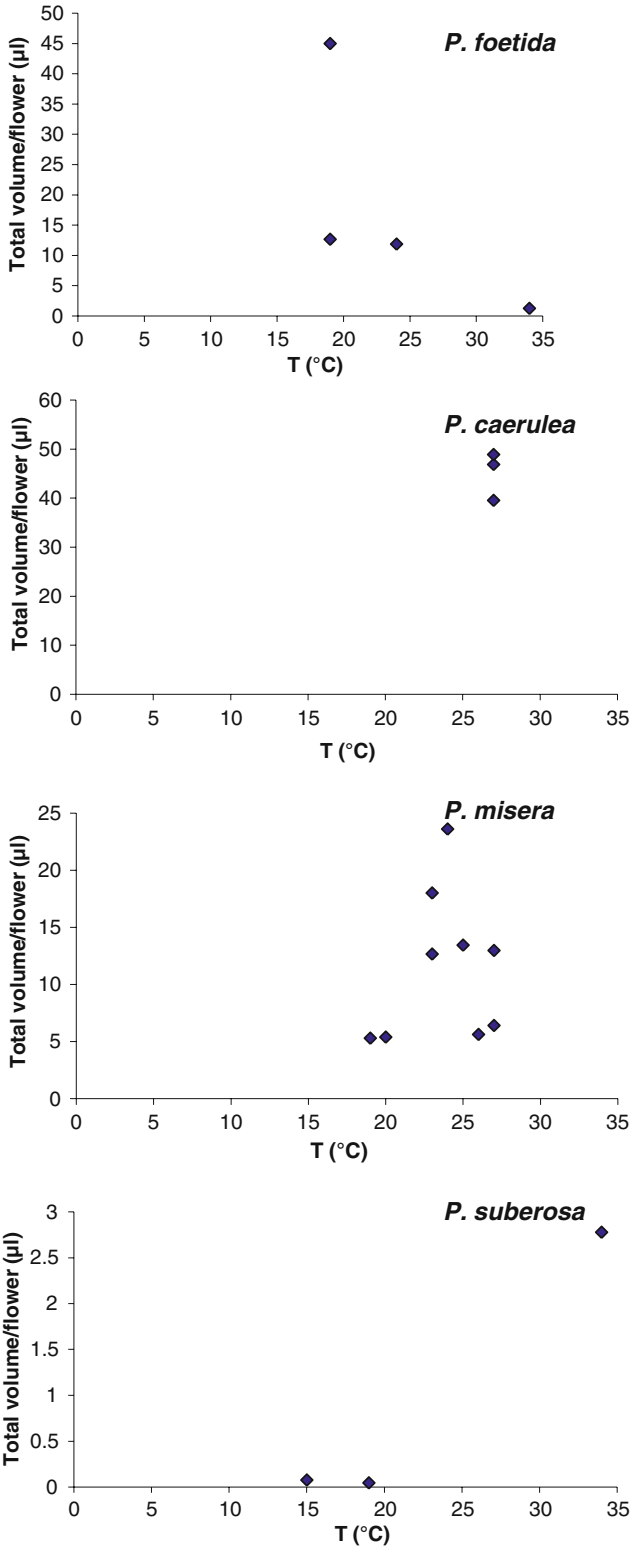

Fig. 4 Total volume of nectar per flower as a function of temperature

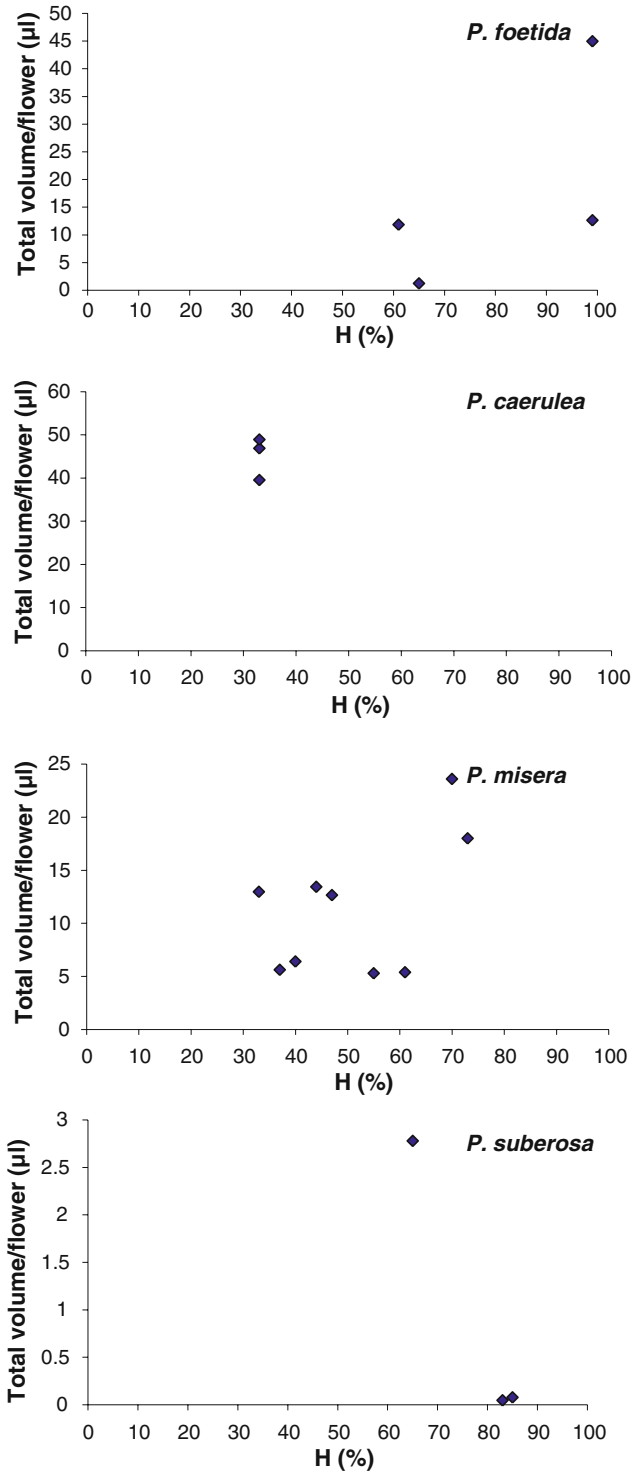

Fig. 5 Total volume of nectar per flower as a function of humidity

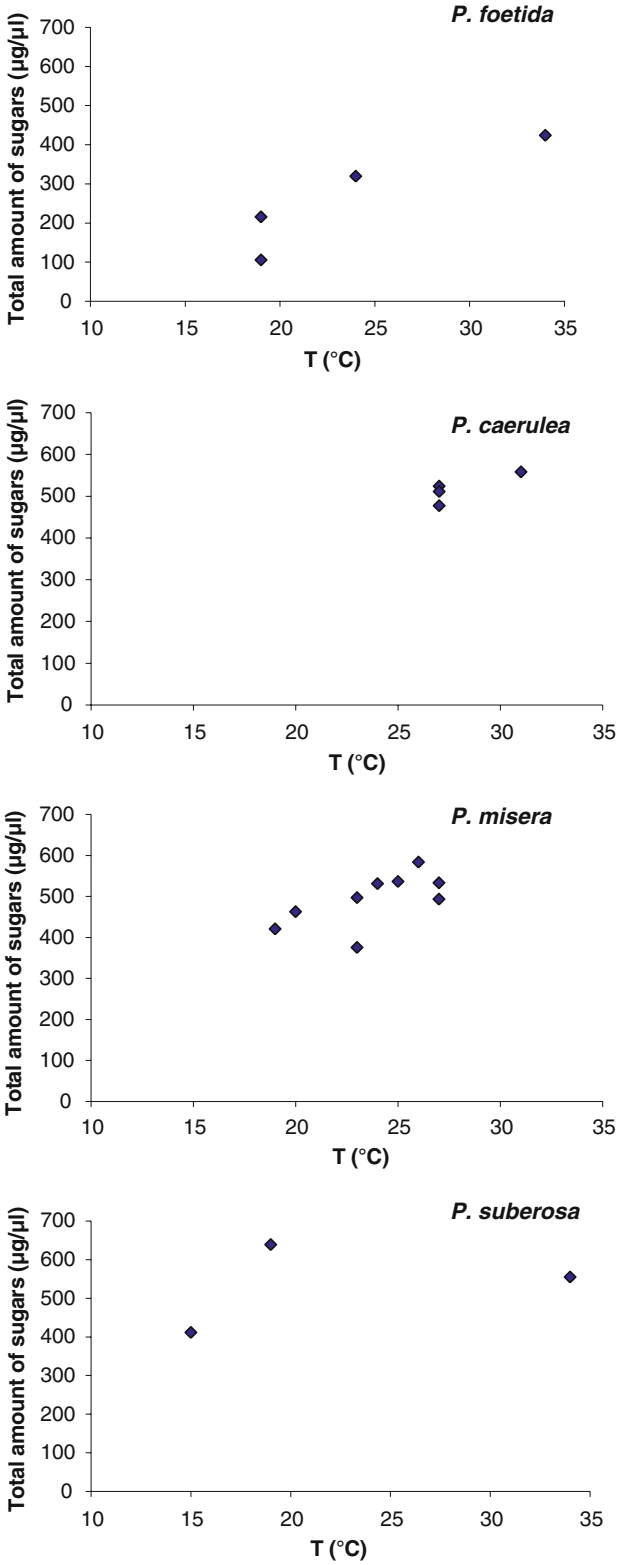

Fig. 6 Total sugar amount per flower as a function of temperature

composition with age have been reported for *Hibiscus syriaca* (Percival 1961) and *Echinacea purpurea* (Wist and Davis 2006), in which the dominance of sucrose changes to fructose in the first case and the dominance of hexoses decreases in the second (in both cases the three main sugars were present in the floral nectar). In turn, sugar ratio varied with varying corolla length in species of *Lycium* (Galetto et al. 1998).

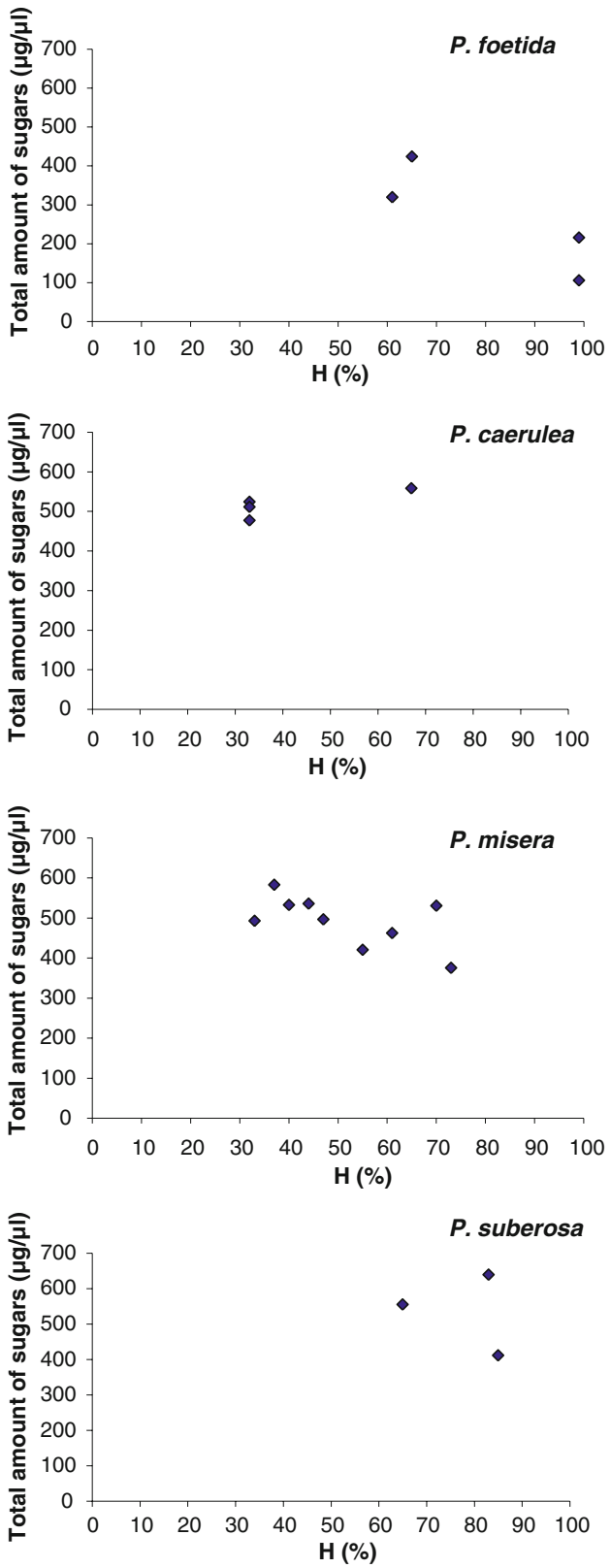

Fig. 7 Total sugar amount per flower as a function of humidity

P. caerulea is pollinated by long-tongued bees (Amela García and Hoc 1997), *P. foetida* and *P. misera* are pollinated by short-tongued bees (Gottsberger et al. 1988; Amela García 1999), and *P. suberosa* is pollinated by short-tongued bees and wasps (Koschnitzke and Sazima 1997). Taking in account the length of the glossa of the main pollinators, the apparent correlation between glossa length and nectar type licked with regard to sugar preponderance (Baker and Baker 1983b) is not supported by these data; the only approximations might be the sucrose-dominant *P. suberosa*, as wasp flowers tend to be richer in sucrose than short-tongued bee flowers (Baker and Baker 1983b) and the hexose-rich *P. foetida*. These authors attributed the relationship to evolutionary constraints of the five plant families analysed rather than to a real correlation between glossa length and sugar preponderance. Those correlations are maintained for some cases, such as the high-sucrose nectar of hummingbird and hawkmoth-pollinated species (Freeman and Worthington 1985), the hexose-rich nectar of the male flowers of *Euterpe precatoria* visited by beetles and bees (Küchmeister et al. 1997), the hexose-dominant nectar of *Symphonia globulifera* pollinated mainly by perching birds (Gill et al. 1998), the hexose-rich nectar of *Silene* species often visited by bumblebees (Witt et al.1999), and the sucrose-dominated nectar of some sphingophilous species (Witt et al. 1999). Perret et al. (2001) did not find a strong tendency among the tribe examined and attributed plant–pollinator relationships to flower display rather than to nectar characteristics. Galetto et al. (1998) considered nectar chemistry a conservative characteristic and Galetto and Bernardello (2004) suggested that nectar traits were determined by structural constraints in the genus they explored. A slight but not significant tendency among the Aculeata (short-tongued Hymenoptera preferred lower sucrose/hexose ratios) was encountered by Petanidou (2005). Although the correspondence between pollinator type and sugar concentration (Cruden et al. 1983) or between pollinator type and sugar relative proportion (Baker and Baker 1983a) often occurs, insects usually exhibit other preferences when artificial liquids are offered to them (Gardener et al. 2003). This reveals that other factors, for example structural constraints, resource availability, presence of competitors or predators, are important in nature when insects visit flowers.

Amino acids

The composition, number, and total quantity of amino acids exhibited strong intraspecific variation; variation was even substantial within a single plant. Such variability has

Table 3 Effect of different meteorological conditions on sugar ratio

Species/sample	Sucrose/hexoses ratio	*T* (°C)	*H* (%)	Rain	Irradiance	Air movement
P. foetida						
1	0.39	34	65	No	Sun	Calm
3	0.13	19	99	Yes	Sun	Calm
5	0.20	24	61	No	Sun	Calm
6	0.11	19	99	Yes	Cloudy	Calm
P. caerulea						
25	0.29	27	33	No	Sun	Calm
27	0.34	27	33	No	Sun	Calm
28	0.29	27	33	No	Sun	Calm
82	0.67	31	67	No	Sun	Calm
P. misera						
23		27	33	No	Sun	Calm
72	30.52	19	55	No	Sun	Calm
73	42.94	27	40	No	Sun	Calm
74	23.81	20	61	No	Sun	Windy
75	51.36	26	37	No	Sun	Windy
76	35.33	23	47	No	Sun	Windy
77	43.85	25	44	No	Sun	Windy
79	32.96	23	73	No	Sun	Windy
80	44.48	24	70	No	Sun	Windy

T temperature, *H* humidity

been reported for nectar of other species (Gottsberger et al. 1989, Gardener and Gillman 2001, Nicolson 2007). Although a clear tendency could not be detected, the total amount of amino acids seems to vary through anthesis for each species. A variation in the presence of certain amino acids with floral longevity was also outlined. Temporal variation of amino acid quantity and quality was detected by Gottsberger et al. (1990), Stpiczýnska (2001), and Piechowski (2007); the first authors registered an increase in the total amount and number with time, the second authors detected an increment in amino acid content during anthesis in the ten day-lasting flowers of an orchid, but the oldest flowers showed a sharp decrease, and the last author discovered a decrease to half of the total concentration and of the dominant amino acid with floral lifespan.

The lower total amount of amino acids in stage 3 compared with stage 2 in some species suggests resorption. This phenomenon occurs with sugars (Búrquez and Corbet 1991; Pedersen et al. 1958 cited in Nepi and Stpiczýnska 2008), amino acids (Ziegler and Lüttge 1959 cited in Nepi and Stpiczýnska 2008) and with both (Kartashove and Tsytlenok 1968 cited in Nepi and Stpiczýnska 2008), but not in all the species tested (Pleasants 1983; Nepi et al. 2001; Stpiczýnska and Pielecki 2002).

The total quantity of amino acids in the *Passiflora* species studied was higher than most figures reported for other floral nectars (Gottsberger et al. 1984, 1989, 1990; Piechowski 2007); the high concentration resembles that observed for *Caryocar brasiliense, Musa* cf. *Ensete* and *Erythrina crista-galli*, pollinated by bats, Old-World birds, and hummingbirds, respectively (Gottsberger et al. 1984), out of 32 species analysed by these authors; these species appear outliers and not representative of the average calculated with the rest of the species with an equivalent pollination syndrome. The total amount of amino acids of every *Passiflora* species surpassed known values for bee and wasp-pollinated flowers; compared with the figures given by Baker and Baker (1983a, b, 1975), it fell in the range between beetle and specialist flies for *P. foetida* and *P. caerulea* and in the range of specialist flies for *P. misera* and *P. suberosa*. Lack of correspondence with the values expected for certain pollinators and amino acid concentrations was also noted by Gottsberger et al. (1989). The "known values" were mainly provided by Baker and Baker (1986), on the basis of an average of five previous works in which only recently opened flowers were sampled. Owing to the enormous intraspecific, intraplant, and even intrafloral (interfloral stage) variability, investigations with an ecological focus are needed. This viewpoint should also consider the particular (Gardener et al. 2003) or versatile (Mevy-Schütz and Erhardt 2003) preferences of nectarivores.

The range of numbers of amino acids fits with the figures of proteinogenic amino acids usually found in floral nectars (Baker and Baker 1973; Toledo and Hernández 1979; Hernández 1981; Piechowski 2007). Of the amino acids required by insects (Koptur 1992), arginine, histidine, and threonine were present in most of the samples from the

Table 4 Amino acid profiles of floral nectar of *Passiflora* species (ng/μl)

S	P	F	Asp	Ser	Glu	Gly	His	Arg	Thr	Ala	Pro	Cys	Tyr	Val	Met	Lys	Ile	Leu	Phe	Total
P. foetida																				
1	6	3	0	0	0	0	35.23	77.19	16.19	146.43	163.86	0	0	0	0	0	0	0	0	438.89
3	6	3	0	0	0	0	0	0	0	44.86	358.17	0	0	0	0	0	0	0	0	403.03
5	6	2	87.69	18.28	16.57	7.035	27.51	26.06	19.67	150.63	293.38	0	0	8.87	0	11.39	5.10	0	0	672.18
6	6	2	13.43	29.51	4.15	24.27	41.81	13.52	1.46	114.54	988.26	0	1.70	20.38	0	0	18.30	10.69	7.53	1289.56
31	5	3	79.49	52.54	98.81	37.23	211.30	98.25	79.96	67.86	532.10	0	89.66	101.43	0	45.30	98.89	94.13	109.58	1796.54
36	7	3	0	0	0	0	369.26	0	290.39	223.16	621.30	0	0	0	0	238.95	0	0	0	1743.06
37	4	3	0	70.54	0	0	125.21	139.04	101.21	209.98	304.74	0	0	78.36	0	82.35	93.42	0	0	1204.83
39	37	3	0	184.30	176.73	0	420.68	185.90	139.26	124.37	1208.26	0	0	157.95	0	95.04	168.37	168.41	206.24	3235.50
45	3	3	0	131.26	177.90	0	205.21	202.36	147.23	186.47	1297.36	0	0	114.28	0	81.31	130.19	0	0	2673.56
48	9	3	0	76.76	97.08	0	189.63	108.07	83.55	50.30	705.96	0	93.03	77.69	0	45.98	80.70	81.90	98.50	1789.13
49	11	3	0	68.62	0	0	172.66	80.81	63.63	44.76	537.87	0	0	69.26	0	40.15	71.73	72.63	85.82	1307.92
P. caerulea																				
27	1	1	1	143.90	149.98	0	414.24	227.65	166.05	99.67	1198.29	0	0	110.62	0	106.87	0	0	187.29	2805.55
28	1	2	0	99.51	0	0	186.22	170.60	126.29	158.70	695.90	0	0	0	0	0	0	0	0	1437.21
63	1	3	33.88	59.81	46.67	0	85.78	81.16	57.81	67.66	991.63	0	0	30.57	0	22.43	35.50	36.06	47.20	1596.14
78	2	2	0	0	0	0	0	268.67	205.00	213.44	381.96	0	0	0	0	129.58	0	0	0	1198.65
82	2	3	0	80.59	0	0	0	209.60	160.46	161.07	396.56	0	0	0	0	81.92	0	0	0	1090.21
129	3	2	0	90.98	112.35	0	162.41	207.46	168.96	86.36	1171.95	0	0	0	0	0	0	0	0	2000.47
133	3	1	0	86.09	110.67	0	102.28	146.86	115.07	66.96	794.43	0	0	69.71	0	49.93	0	0	0	1541.99
P. suberosa																				
2	4	2	0	0	129.08	47.42	345.15	286.52	198.77	27.04	1009.94	0	0	0	0	0	0	0	0	2043.94
7	5	3	0	0	0	0	0	0	0	34.85	57.19	0	0	0	0	0	0	0	0	92.04
81	Hr6	3	0	223.74	0	0	463.31	647.41	522.53	205.57	1810.88	0	0	229.68	0	161.76	291.75	313.01	0	4869.65
84	44	3	108.47	101.42	110.56	0	191.73	277.11	238.43	82.81	980.30	0	0	78.35	0	0	96.54	0	0	2265.73
88	45	2	207.10	226.55	307.22	0	514.43	392.66	312.36	229.23	2579.16	0	0	146.81	0	108.75	176.29	0	0	5200.56
91	45	3	0	184.25	332.80	0	498.55	467.77	388.39	162.11	2262.24	0	0	0	0	102.07	162.48	0	0	4560.66
P. misera																				
23	1	3	121.56	113.37	149.67	0	272.50	353.60	293.73	92.28	1045.85	0	0	82.58	0	52.98	90.58	90.66	114.90	2874.25
72	1	1	0	91.35	160.09	0	183.56	312.60	259.27	97.78	823.08	0	0	82.56	0	96.20	0	0	0	2106.48
77	1	2	95.89	89.21	175.46	0	182.22	458.33	399.80	98.70	1483.43	0	0	67.98	82.95	50.59	0	0	0	3184.54
93	3	3	72.59	63.08	70.86	0	100.51	187.64	163.50	45.16	475.19	0	0	54.67	0	60.94	62.08	66.16	78.66	1501.04

F floral stage, *P* plant, *S* sample

Table 5 Quantitative data for amino acids in the floral nectar of *Passiflora* spp.

Species	*P. foetida*	*P. caerulea*	*P. misera*	*P. suberosa*
Total amount/flower X ± SD (ng/µl)	1504.93 ± 883.31	1667.17 ± 582.09	2416.57 ± 760.17	3172.09 ± 2025.12
Number of amino acids	2–15	5–13	9–13	2–11
Total amount/floral stage X ± SE (ng/µl)	1 2 3 – 980.87 ± 436.55 1621.38 ± 931.43	1 2 3 2173.77 ± 893.47 1545.44 ± 411.72 1343.17 ± 357.75	1 2 3 2106.48 3184.54 2187.64 ± 971.00	1 2 3 – 3622.25 ± 2232.07 2947.22 ± 2229.75
Flowers (*n*)	11	7	4	6
Plants (*n*)	8	3	2	5

1, 2, and 3 denote the staminate, staminate-pistillate, and staminate floral stages, respectively

– denotes no data available

four species and in high concentration, whereas isoleucine, leucine, and valine appeared in some of the samples from the four species; lysine was more variable in appearance between the species, methionine was absent in all but one sample of *P. misera*; phenylalanine was present in a few samples of three species but in none of *P. suberosa*; threonine was detected only in some samples of *P. foetida*. Of the "quasi essential" amino acids (Gottsberger et al. 1984), alanine was present in all the samples (i.e., in all the floral stages) in all the species in great abundance, serine was detectable in most of the samples of the whole species, glycine was absent from *P. misera* and *P. caerulea* and appeared in a few samples of *P. suberosa* and *P. foetida*. Asparagine and glutamic acid occurred in all the samples of *P. misera* and in some of the other species; cysteine did not occur in any species. Proline was the most abundant in all the samples (i.e., in all the floral stages) in all the species and it was omnipresent. Such abundance has also been reported for other floral nectars (Blüthgen et al. 2004; Piechowski 2007; Carter et al. 2006) and has been interpreted as an attractant because at least honeybees preferred synthetic nectars rich in this amino acid (Carter et al. 2006).

Nectar and weather conditions

Post-secretory changes in concentration are mainly influenced by microclimatic effects (Corbet et al. 1979); this is particularly evident in exposed nectars, whose concentration increased because of evaporation (Plowright 1981, 1985); accordingly, sugar concentration was negatively correlated with corolla length in *Lycium* spp. (Galetto et al. 1998). But sugar production may be affected by weather even in tubular flowers—sugar secretion rate was significantly higher on sunny days than on overcast days (Pleasants 1983); this change was not because of evaporation in the tubular flowers sampled and relative humidity was discounted as an influence, the responsibility being attributed to temperature and sunlight (Pleasants 1983 and literature cited therein). The rate of nectar secretion and the amount of sugar production was augmented by increasing temperature and decreasing humidity in *Caesalpinia pulcherrima* (Cruden et al. 1983).

A trend to a maximum total amount of sugars with increasing temperature and decreasing humidity was observed in this study, but sugar concentration measured with a hand-refractometer was constant throughout anthesis and with varying meteorological conditions in different *Passiflora* species; even rain cannot dilute this concealed resource in this genus (Amela García 1999). The lack of correlation of nectar volume or amino acid concentration with temperature or humidity, and the constancy of sugar proportion with distinct weather conditions in the species

Table 6 Effect of temperature (*T*) and humidity (*H*) on amino acid concentrations in floral nectar of some *Passiflora* species

Species	Sample	Total amino acid amount (ng/μl)	*T* (°C)	*H* (%)	Irradiance	Rain	Air movement
P. foetida	6	1289.558	19	99	Cloudy	Yes	Calm
	3	403.026	19	99	–	Yes	Calm
	5	672.176	24	61	Sunny	No	Calm
	1	438.89	34	65	–	–	–
P. caerulea	27	2805.552	27	33	Sunny	No	Calm
	28	1437.211	27	33	Sunny	No	Calm
	78	1198.651	30	29	Sunny	No	Windy
	82	1090.206	31	67	Sunny	No	Calm
P. misera	72	2106.475	18	58	Sunny	No	Calm
	77	3184.535	25	44	Sunny	No	Windy
	23	2874.245	27	33	Sunny	No	Calm

– , not recorded

studied is probably because of concealment of the nectar in the *Passiflora* flowers.

Nectar chemistry and taxonomy

According to the old classification of Killip (1938), *P. caerulea* belongs to subgenus *Passiflora*, *P. foetida* to subgenus *Dysosmia* and *P. misera* and *P. suberosa* to subgenus *Decaloba.* Feuillet and MacDougal (2004) proposed combining the subgenera *Passiflora* and *Dysosmia*. The affinity between *P. foetida* and *P. caerulea* versus *P. misera* and *P. suberosa* is reflected by their nectar chemistry. There was a similarity between the total amount of amino acids in the first pair compared with the second pair.

The significant difference in the amount of each sugar between *P. foetida* and *P. caerulea* is in accordance with the inclusion of these species in different sections of subgenus *Passiflora* by Feuillet and MacDougal (2004). With regard to the sugars ratio, *P. caerulea* and *P. foetida* showed hexose-rich nectars, whereas *P. misera* and *P. suberosa* showed sucrose-dominated nectars. So the composition and proportion of the sugars of the species belonging to the same subgenus (according to the new classification), is more similar than that for the species grouped in a different subgenus. Other species included in subgenus *Decaloba* were also sucrose-dominant—three species examined by Durkee et al. (1981) and *P. gracilis* (Percival 1961), a species that belongs to the same Killip's infrageneric category (section) as *P. suberosa*. Nevertheless, species belonging to subgenus *Passiflora* analysed by other authors were sucrose-dominant, in contrast with those examined here: *P. quadrangularis* (now *P. alata*), *P. subpeltata* (Percival 1961), and *P. alata* (Gottsberger et al. 1984). Thus, subgenus *Decaloba* seems to be more homogeneous with regard to this characteristic. Considering, however, that the genus includes more than 500 species (Feuillet and MacDougal 2004), much more research must be conducted before drawing definite conclusions.

Acknowledgments Thanks to D. Piechowski, who kindly gave advice for concentration conversions, to H. Malchus, who analysed the samples, to M.L. Bolkovic for her expertise teaching use of Excel's graphics, and to L. Rodríguez Planes for help with statistics. This work was financed by the grant PICT 32866 of the ANPCyT (Agencia Nacional de Promoción Científica y Tecnológica) from Argentina. M.T. Amela García is a research assistant from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas).

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