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Floral Nectaries, Nectar Production Dynamics and Chemical Composition in Six *Ipomoea* Species (Convolvulaceae) in Relation to Pollinators

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• *Background and Aims* Floral nectaries and nectar features were compared between six Argentinian *Ipomoea* species with differences in their pollinator guilds: *I. alba*, *I. rubriflora*, *I. cairica*, *I. hieronymi* var. *hieronymi*, *I. indica*, and *I. purpurea*.
• *Methods* Pollinators were recorded in natural populations. The morpho-anatomical study was carried out through scanning electron and light microscopy. Nectar sugars were identified via gas chromatography. Nectar production and the effect of its removal on total nectar sugar amount were determined by using sets of bagged flowers.
• *Key Results* Hymenopterans were visitors of most species, while hummingbirds visited *I. rubriflora* and sphingids *I. alba*. All the species had a vascularized discoidal nectary surrounding the ovary base with numerous open stomata with a species-specific distribution. All nectar samples contained amino acids and sugars. Most species had sucrose-dominant nectars. Flowers lasted a few hours. Mean nectar sugar concentration throughout the lifetime of the flower ranged from 34·28 to 39·42 %, except for *I. cairica* (49·25 %) and *I. rubriflora* (25·18 %). *Ipomoea alba* had the highest nectar volume secreted per flower (50·12 μL), while in the other taxa it ranged from 2·42 to 12·00 μL. Nectar secretion began as soon as the flowers opened and lasted for a few hours (in *I. purpurea*, *I. rubriflora*) or it was continuous during the lifetime of the flower (in the remaining species). There was an increase of total sugar production after removals in *I. cairica*, *I. indica* and *I. purpurea*, whereas in *I. alba* and *I. rubriflora* removals had no effect, and in *I. hieronymi* there was a decrease in total sugar production.
• *Conclusions* The chemical composition, production dynamics and removal effects of nectar could not be related to the pollinator guild of these species. Flower length was correlated with nectary size and total volume of nectar secreted, suggesting that structural constraints may play a major role in the determination of nectar traits of these species.

Key words: Nectary structure, nectar chemical composition, nectar production dynamics, nectar removal effects, pollinators, *Ipomoea*, Convolvulaceae, morning glory, Argentina.

INTRODUCTION

Floral nectar is widely known as the key reward offered by animal-pollinated plants to their pollen vectors (Proctor *et al*., 1996). This exudate is secreted by nectaries, i.e. glandular tissues located on various floral parts whose features are significant in plant taxonomy and phylogeny (Fahn, 1979). Sugars dominate the total solutes in floral nectar: these are mainly sucrose, fructose and glucose in varying proportions according to the species (Baker and Baker, 1983*a*, *b*; Freeman *et al*., 1991; Stiles and Freeman, 1993). Other compounds, such as amino acids, phenols, lipids and antioxidants, are found as well, but mostly in trace quantities (Baker and Baker, 1975, 1983*a*). All these substances often impart a particular taste and odour that may be essential for maintaining certain pollinator groups (Southwick, 1990). In many cases it has been interpreted that pollinators determine nectar components and, thus, the nectar sugar ratios together with flower and inflorescence morphology may be good predictors of the pollinators (cf. Baker and Baker, 1990). For instance, hummingbird- and hawk-moth-pollinated flowers tend to produce sucrose-dominant nectar, whereas bee-pollinated flowers tend to produce nectars with a predominance of hexose (Baker and Baker, 1983*a*, *b*). In addition, experimental studies on sugar preferences of hummingbirds have demonstrated that they preferred sucrose solutions instead of equivalent monosaccharide ones (e.g. Martínez del Río, 1990; Stromberg and Johnsen, 1990). However, in other instances nectar composition may be a conservative character due to phylogenetic constraints (cf. Galetto *et al*., 1998).

Nectar is secreted with particular rhythms, throughout the lifespan of a flower, which allow the nectar production dynamics of a species to be determined. Knowledge of nectar production dynamics is fundamental to the understanding of the plant–animal relationship; aspects such as the plant's strategy of offering nectar, the activity patterns, frequency and diversity of pollinators of a plant species, the rates of nectar consumption by animals, among others, could not be understood without it. Nectar production may show diverse patterns according to the different guilds of pollinators that visit the flowers (e.g. Feinsinger, 1978; Cruden *et al*., 1983; Galetto and Bernardello, 1992), leading to the assumption that there are coevolutionary relationships between nectar traits and pollinator type (Baker and Baker, 1983*a*, *b*). For instance, hawk-moth-pollinated flowers produce abundant nectar with low concentration values and

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bee-pollinated flowers secrete compartively less nectar with higher concentrations, whereas hummingbird-pollinated flowers show intermediate values (e.g. Pyke and Waser, 1981; Opler, 1983; Baker and Baker, 1983*a*; Sutherland and Vickery, 1993).

At the same time, the effect of nectar removal by floral visitors may have a pronounced effect on the total amount secreted by a flower. Although in some species removal does not modify nectar production (e.g. Galetto and Bernardello, 1993, 1995; Galetto *et al*., 2000), in others the total amount of sugar in the nectar may be either increased (e.g. Pyke, 1991; Galetto and Bernardello, 1995; Castellanos *et al*., 2002) or decreased (e.g. Galetto and Bernardello, 1992; Bernardello *et al*., 1994; Galetto *et al*., 1997). Predictions for these patterns are not straightforward because they may be related to pollinators, environmental factors, plant resource allocation, or other factors.

Six sympatrically occurring *Ipomoea* species that have differences in the pollinator guilds, floral colours and breeding systems were chosen to examine their floral nectaries, nectar components and nectar production dynamics to evaluate if there are correlations among these features and to consider the results in the context of plant–pollinator interactions. *Ipomoea* (whose species are commonly known as 'morning glory') is a cosmopolitan climbing genus from warm and pantropical regions with approx. 650 species (Austin and Huáman, 1996) with large showy flowers that are easy to manipulate. Its members have trumpet-shaped flowers of different colours—mainly white, purple, blue, pink, red (Cronquist, 1981). These are visited by a diverse array of animals, including bees, hawk moths, beetles, butterflies, long-tongued flies, hummingbirds and bats (e.g. van der Pijl, 1954; Vogel, 1954; Schlising, 1970; Sobreira-Machado and Sazima, 1987; McDonald, 1991). These visitors look for the floral nectar secreted by a discoidal nectary surrounding the ovary base (Fahn, 1979; Cronquist, 1981). In addition, extrafloral nectar and nectaries are widespread in *Ipomoea* in petioles and/or in epals that are mostly visited by ants and serve as a herbivore defence mechanism (Elias, 1983; Keeler and Kaul, 1984).

In spite of the attractiveness of the flowers of this diverse genus and the importance of some species as crops or invasives (Austin and Huáman, 1996), studies on the floral nectar features of the genus are few. Only five taxa have been examined for their floral nectar composition (Keeler, 1977, 1980; Stucky and Beckmann, 1982; Freeman *et al*., 1985, 1991), and only six species have been incidentally examined for their nectar secretion (Real, 1981; Stucky and Beckmann, 1982; Stucky, 1984; Devall and Thien, 1989). The present work was undertaken to study and compare the floral nectaries and nectar features in six Argentinian *Ipomoea* species addressing the following questions: (1) What are the local flower animal visitors? (2) What is the floral nectary structure? (3) What is the chemical composition of the nectar? (4) What are the production dynamics of nectar throughout the lifetime of the flower? (5) What is the floral response to nectar removal? We expected to find differences in nectar sugar composition and production dynamics among the species as they are visited by different pollinator guilds (see above).

The species studied included: *I. alba* (subgen. *Quamoclit*) with long, white, hawk-moth-pollinated flowers (McDonald, 1991), *I. rubriflora* (subgen. *Quamoclit*) with medium-sized, red, allegedly hummingbird-pollinated flowers (cf. Wilson, 1960; Austin, 1975) and *I. cairica* (subgen. *Quamoclit*) with violet-pink flowers, *I. hieronymi* var. *hieronymi* (subgen. *Eriospermum*) with pink flowers, *I. indica* (subgen. *Ipomoea*) with blue flowers and *I. purpurea* (subgen. *Ipomoea*) with pink, white, or purple flowers, all bee-pollinated (Real, 1981; Maimoni-Rodella *et al*., 1982; Maimoni-Rodella and Rodella, 1992; Pinheiro and Schlindwein, 1998; Galetto *et al*., 2002). *Ipomoea rubriflora* and *I. hieronymi* are endemic to Bolivia and Argentina (Chiarini and Ariza Espinar, 2004), whereas the other species are mainly pantropical (Austin and Huáman, 1996; Chiarini and Ariza Espinar, 2004). Regarding their breeding system, some species are self-compatible (SC), such as *I. alba* (Martin, 1970), *I. purpurea* (Chang and Rausher, 1999; Galetto *et al*., 2002), and *I. rubriflora* (L. Galetto, unpubl. res.), whereas others are self-incompatible (SI), such as *I. hieronymi* (L. Galetto, unpubl. res.), *I. indica* (Martin, 1970), and *I. cairica* (Maimoni-Rodella *et al*., 1982; Pinheiro and Schlindwein, 1998; Laporta and Suyama, 2002).

MATERIALS AND METHODS

The source of the populations studied for each analysis is given in Table 1. In each population three to five individuals were sampled.

In each population studied, flower visitors were recorded on individual plants at the middle of the lifetime of the flower, for 15 min on three different days.

To analyse nectary structure, flowers were fixed in 70 % ethanol, dehydrated in an ethyl alcohol–xylol series, and embedded in Paraplast. Cross- and longitudinal sections were cut at 10 μm, mounted serially, stained with safranin–astral blue (Maacz and Vagas, 1961), and observed with a compound microscope at ×100–1000 magnifications. To detect stomata in the nectariferous tissue, glands were cleared with standard bleach for 1 min and stained with Lugol solution (I_2/IK). Photomicrographs were taken with Kodak T-Max film, 100 ASA, with an Axiophot-photomicrographic system equipped with automatic exposure.

Flower length, excluding the pedicel, was measured (n = 10 flowers per species). Nectary tissue volume was calculated with the non-circular section toroids' formula: $V = 2\pi sr$, where s = nectary sectional area and r = nectary radius measured from the sections' centre of gravity (n = 4 flowers per species). This parameter was estimated with reference to the weight of the drawings of the nectary in longitudinal section (the two stained areas at the ovary base). The drawings were made on a homogeneous paper using a camera lucida fitted on a stereomicroscope.

Ovaries for observation under a scanning electron microscope (SEM) were dehydrated in an acetone series and dried using CO_2 in a critical-point dryer (Balzers, Switzerland).

TABLE 1. *Species and study sites for* Ipomoea *populations from Argentina, Prov. Córdoba*

Species	Population	Flower colour	Localities and dates of study	Voucher	Data taken
I. alba L.	1	White	Dept Capital, Barrio Cofico, January 13, 1996.	Bernardello & Galetto 890	Nectar, pollinators
	2	White	Dept. Capital, Barrio Cerro de las Rosas, December 12, 1987.	Galetto & Bernardello 10	Nectar chemistry, nectary, pollinators
	3	White	Dept. Capital, Argüello, February 22, 1989.	Galetto & Bernardello 49	Nectar chemistry, pollinators
I. cairica (L.) Sweet	1	Violet-pink	Dept. Colón, Villa Allende, November 11, 1987.	Galetto & Bernardello 8	Nectar chemistry, nectary, pollinators
	2	Violet-pink	Dept. Colón, El Diquecito, November 21, 1987.	Galetto & Bernardello 3	Nectar chemistry, pollinators
	3	Violet-pink	Dept. Colón, La Quebrada, October 26, 1989.	Galetto w.n.	Nectar chemistry, pollinators
	4	Violet-pink	Dept. Colón, Río Carnero, February 13, 1993.	Galetto w.n.	Nectar, pollinators
	5	Violet-pink	Dept. Colón, Río Ceballos, 20 Dec. 1991.	Galetto w.n.	Nectar chemistry
I. hieronymi (O.K.) O'Donell var. *hieronymi*	1	Pink	Dept. Capital, Villa Warcalde, 30 Dec. 1988.	Galetto 38	Nectar chemistry, nectary, pollinators
	2	Pink	Dept. Santa María, Los Aromos, 29 Dec. 1996	Galetto 717	Nectar, pollinators
I. indica (Burm. f.) Merr.	1	Blue	Dept San Justo, Miramar, 24 May 1995	Galetto & Bernardello 322	Nectar secretion, pollinators
	2	Blue	Dept Capital, Villa Warcalde, 30 Dec. 1988	Galetto & Bernardello 40	Nectar chemistry, nectary, pollinators
	3	Blue	Dept Capital, Barrio Escobar, 19 Dec. 1987	Galetto & Bernardello 11	Nectar chemistry, pollinators
	4	Blue	Dept Capital, Cerro de Las Rosas, 21 Dec. 1988	Galetto & Bernardello 35	Nectar chemistry
I. purpurea (L.) Roth	1	Purple	Dept Santa María, La Serranita, 7 Feb. 1997	Galetto 671	Nectar, pollinators
	2	Pink	Dept Capital, Córdoba, 8 June 1997	Galetto 725	Nectar, pollinators
	3	Purple	Dept Capital, Córdoba, 8 June 1997	Galetto 726	Nectar, pollinators
	4	White	Dept Capital, Barrio Escobar, 5 Apr. 1988	Galetto & Bernardello 12	Nectar chemistry, nectary
	5	Purple	Dept Capital, Barrio Escobar, 7 Apr. 1988	Galetto & Bernardello 13	Nectar chemistry, pollinators
I. rubriflora O'Donell	1	Red	Dept Punilla, Carlos Paz, 22 Mar. 1989	Galetto 52	Nectar chemistry, nectary
	2	Red	Dept Santa María, La Serranita, 16 Mar. 1997	Galetto 714	Nectar secretion and chemistry
	3	Red	Dept Colón, Río Carnero, 13 Feb. 1993	Galetto w.n.	Nectar secretion and chemistry

Vouchers are deposited at CORD (Museo Botanico de Córdoba).
w.n., Without number.

Dried samples were mounted and then gold-coated to a thickness of 25 nm (Balzers). Photomicrographs were taken with a Jeol 35CF scanning electron Photomicroscope on AgfaPan APX 100.

In the field, nectar drops for each sample were placed in 1 mL vials and quickly frozen. Sugar concentration and nectar volume were measured in the field with an Atago pocket refractometer and graduated capillary glass tubes, respectively. Tests following Baker and Baker (1975) for amino acids, lipids, phenols, alkaloids and reducing acids were made on nectar spots on chromatography paper. The 'histidine scale' (Baker and Baker, 1975) was used to quantify amino acids. Sugars were identified via gas chromatography. Nectar was lyophilized and silylated according to Sweeley *et al*. (1963). The derivatives were then injected into a Konik KNK 3000-HRGS gas chromatograph equipped with a Spectra-Physics SP 4290 data integrator, a flame ionization detector and an OV 101 column (2 m long and 3 mm diameter, on 3 % Chromosorb G/AW-DMCS mesh 100–120). Nitrogen was the carrier gas and the following temperature programme was used: 208 °C/2 min, 1 °C/min until 215 °C, 8 °C/min until 280 °C for 5 min. Carbohydrate standards (Sigma Chemical, St Louis, MO, USA) were prepared using the same method. Chromatographic sugar analyses were run at least twice for each sample. Sugar ratios, r = sucrose [S] : (fructose [F] + glucose [G]), and hexose ratios, h = G : F, were calculated as per Baker and Baker (1983*b*).

Floral longevity was determined in ten bagged flowers by following the flowers' development until the corollas began to wilt. Randomly chosen flowers in the bud stage were bagged using paper bags to prevent pollinator visits and were tagged for identification. Nectar production was determined by using flower sets of seven to 20 flowers each according to flower availability. Flowers of a set were assigned from different individuals (one to four per each plant according to the availability of plants). The sampling schedule took into account the lifetime of the flower of each species, with either four or five flower sets (Table 4). Data were taken once for each set, allowing the nectar to accumulate until it was measured. Net nectar production rate (NPR) per hour was calculated as: mg of sugar produced between measurements/number of hours between them (mg h^{-1}).

To evaluate the effect of removal on total sugar amount, nectar was removed and measured from the same flower repeatedly during the entire active secretion period. Nectar was extracted with capillary glass tubes without removing the flowers from the plant, taking extreme care to avoid damage to the nectaries. Sets of seven to 20 flowers were subjected to a different number of removals according to the secretion period of the species. Flowers of a set were assigned from different individuals (one to four per each plant according to the availability of plants). According to the flower lifetime of the species, four or five flower sets were assigned (see Table 4); for the first measurement, nectar was allowed to gather for approx. 1 h because it was not secreted in buds, and an interval of approx. 3 h was left to allow nectar to accumulate between measurements. The general scheme was to allow nectar to accumulate for a determined period (approx. 3, 6, 9, 12 h, according to the set; see Table 4) and then to remove it a number of times: set 1 = five to four nectar removals; set 2 = four to three removals; set 3 = three to two removals; set 4 of the species that have five sets = two removals; set 4 and set 5 (control sets, Table 4) = nectar was allowed to accumulate during the entire flower lifetime and only one measurement was performed. The total amount of sugar per flower was calculated as the product of nectar volume and sugar concentration per unit volume, e.g. mg per μL after Bolten *et al*. (1979).

Statistical tests were performed using methods described in Sokal and Rohlf (1995) with the SPSS statistical program package (SPSS release 10.0, 1999). All distributions were tested for randomness of nominal data (Runs test), homogeneity of variances (Levene test), and departures from normality (Kolmogorov–Smirnov for goodness-of-fit test). The effects of nectar removal on the total amount of sugar produced by each set of flowers were compared with one-way analysis of variance (ANOVA) and with the Bonferroni's *post hoc* test for multiple comparisons among pairs of means, to evaluate the consequences of pollinator visits to each species. Regression analyses were done using species means to estimate if flower traits are explained by the increase of flower size. The relationship between pollination guilds and nectar traits was assessed by qualitative comparisons because of the low number of hummingbird and hawk-moth species.

RESULTS

Floral visitors

Hymenopterans were regular visitors of *I. cairica*, *I. hieronymi*, *I. indica* and *I. purpurea* (Table 2). The introduced European bee (*Apis mellifera*) was occasionally observed on the study species, but most visits corresponded to native bees from the families Apidae (*Bombus opifex*, *B. morio*), Megachilidae (*Megachile* sp.), Anthophoridae (*Centris* sp., *Thygater* sp.) and Halictidae (*Anglochloropsis* sp.) (Table 2). In the populations of *I. cairica* and *I. purpurea* studied, both *Bombus* species were more frequent visitors than the other bees. On the other hand, hummingbirds [Trochilidae, both sexes of *Chlorostilbon aureoventris* and females of *Sappho sparganura*] were the most frequent visitors of *I. rubriflora* and sphingids (Sphingidae: *Manduca* sp. and *Agrius cingulata*] of *I. alba* (Table 2).

Floral nectaries

All the species studied had a conspicuous floral (also called nuptial), discoidal nectary surrounding the ovary base (Figs 1A and C; 2A, B, D, E and G). The secretory tissue was composed of intensely stained cells, each with a big nucleus and many small vacuoles (Fig. 2C, F and I), and was supplied by vascular bundles with both xylem and phloem branches (Fig. 2A and H–I). The epithelial cells possessed a cuticle, fewer cellular contents, and generally a big vacuole (Fig. 2I). Numerous, always open, stomata were found on the epidermis of the nectaries (Fig. 1B and D); nectar exudation possibly occurs through them. Their distribution varied according to the species: homogeneously distributed over all the nectary surface (*I. indica* and *I. rubriflora*, Fig. 1A and B), in two areas of the nectary: the apex and in the base (*I. alba*), or exclusively in the apical region (*I. cairica*, *I. hieronymi* and *I. purpurea*; Fig. 1C and D).

Floral longevity

Flowers lasted less than half a day: from 8–9 h in *I. hieronymi*, approx. 10 h in *I. cairica*, *I. purpurea* and *I. rubriflora* to approx. 12 h in *I. alba* and *I. indica*. With the exception of *I. alba*, whose flowers opened at twilight and faded at sunset or exceptionally at midday, the remaining species had diurnal anthesis, lasting from early morning to the afternoon.

Nectar chemical composition

Alkaloids, phenols, antioxidants and lipids were never detected. On the other hand, all samples had amino acids and sugars in variable concentrations (Table 3). Amino acids were found in concentrations from 2 to 7 on the histidine scale (Table 3). The three most common sugars were always detected and the proportions found for the different samples of each taxon were, in general, homogeneous with the exception of *I. cairica* that showed a great intraspecific variability (Table 3). Most species had sucrose-dominant nectars; only *I. cairica* presented hexose dominant or hexose-rich samples (Table 3). Hexose ratios

TABLE 2. *Flower visitors of Argentinian* Ipomoea *species*

Species	Hymenopterans	Hummingbirds	Sphingids
I. alba	–	–	Sphingidae: *Manduca* sp. ***, *Agrius cingulata***
I. cairica	Apidae: *Bombus opifex****, *B. morio****, *Apis mellifera** Megachilidae: *Megachile* sp.** Anthophoridae: *Centris* sp.**, *Thygater* sp.** Halictidae: *Anglochloropsis* sp.**	–	–
I. hieronymi	Apidae: *Bombus opifex**, *B. morio** Megachilidae: *Megachile* sp.** Anthophoridae: *Centris* sp.*, *Melitoma* sp.***, *Thygater* sp.*** Halictidae: *Anglochloropsis* sp.**	–	–
I. indica	Apidae: *Bombus opifex**, *B. morio****, *Apis mellifera** Anthophoridae: *Thygater* sp.*** Halictidae: *Halictus* sp.**	–	–
I. purpurea	Apidae: *Bombus opifex****, *B. morio****, *B. bellicosus* *, *Apis mellifera** Megachilidae: *Megachile* sp.** Anthophoridae: *Thygater* sp.**, *Ptilothrix* sp.* Halictidae: *Halictus* sp.*	–	–
I. rubriflora	Apidae: *Bombus opifex** Vespidae: *Polystes canadensis**	Trochilidae: *Chlorostilbon aureoventiris****, *Sappho sparganura****	–

Frequency: *, rare, **, common, ***, very common, –, not recorded.

indicated that most species had more glucose than fructose (Table 3).

Nectar production dynamics

Nectar traits varied among the different species examined, but was quite constant for each one. Most species had a mean nectar sugar concentration thoughout the flower lifetime ranging from 34·28 to 39·42 % (*I. alba*: $\bar{x} = 34{\cdot}28\ \% \pm 1{\cdot}68$, *I. hieronymi*: $\bar{x} = 36{\cdot}71\ \% \pm 1{\cdot}65$, *I. indica*: $\bar{x} = 37{\cdot}99\ \% \pm 2{\cdot}18$, *I. purpurea*: $\bar{x} = 39{\cdot}42\ \% \pm 1{\cdot}42$). Extreme values were recorded for *I. cairica* with the most concentrated nectar ($\bar{x}$ = 49·25 % ± 3·27), whereas *I. rubriflora* had the most dilute ($\bar{x} = 25{\cdot}18\ \% \pm 0{\cdot}16$). Total nectar volume of unvisited flowers ranged from 50·12 μL in *I. alba* to 2·42 μL in *I. purpurea*.

In none of the species did the buds secrete nectar. Nectar secretion began as soon as the flowers opened and lasted for a few hours, as in *I. purpurea* and *I. rubriflora*, or was continuous during the whole flower lifetime in the remaining species (Table 4, underlined data on the diagonal). *Ipomoea hieronymi* stood out because there was a notable increase of sugar after midday (Table 4). It should be noted that nectar resorption was never detected for the species studied and that *I. alba* had a cessation period in the second half of the flower lifetime (Table 4). Some differences became evident when comparing the nectar production rate (NPR) between the *Ipomoea* species: the hawk-moth-pollinated *I. alba* showed the highest rate (approx. 2·5 mg h^{-1}) during the active secretion period, whereas the hummingbird-pollinated *I. rubriflora* the lowest rate (approx. 0·15 mg h^{-1}). All the bee-pollinated species (*I. cairica*, *I. hieronymi*, *I. indica* and *I. purpurea*) had a similar NPR (approx. 0·4 mg h^{-1}).

Nectar removal effects

Independently of the effect of removing nectar on total sugar production, *I. purpurea* and *I. rubriflora* ceased to secrete nectar after a few hours, whereas the remaining species continued until the end of the flower lifetime (Table 4). After nectar removal, species showed different responses in terms of total nectar sugar produced (Table 4). In *I. hieronymi*, the total amount of nectar produced by flower sets subjected to removals was lower than control sets ($F_{3,44} = 12{\cdot}53$, $P = 0{\cdot}001$; Table 4), i.e. there was an inhibition of nectar sugar production. On the other hand, there was an increase in nectar sugar production in *I. cairica*, *I. indica* and *I. purpurea* after removals ($F_{3,39} = 13{\cdot}52$, $P = 0{\cdot}001$; $F_{4,29} = 5{\cdot}57$, $P = 0{\cdot}002$ and $F_{3,79} = 2{\cdot}77$, $P = 0{\cdot}05$, $F_{3,39} = 4{\cdot}32$, $P = 0{\cdot}01$, respectively; Table 4), whereas in *I. alba* and *I. rubriflora* removals had no effect on total nectar sugar production ($F_{4,35} = 1{\cdot}04$, $P = 0{\cdot}40$, and $F_{3,79} = 1{\cdot}42$, $P = 0{\cdot}28$, respectively; Table 4).

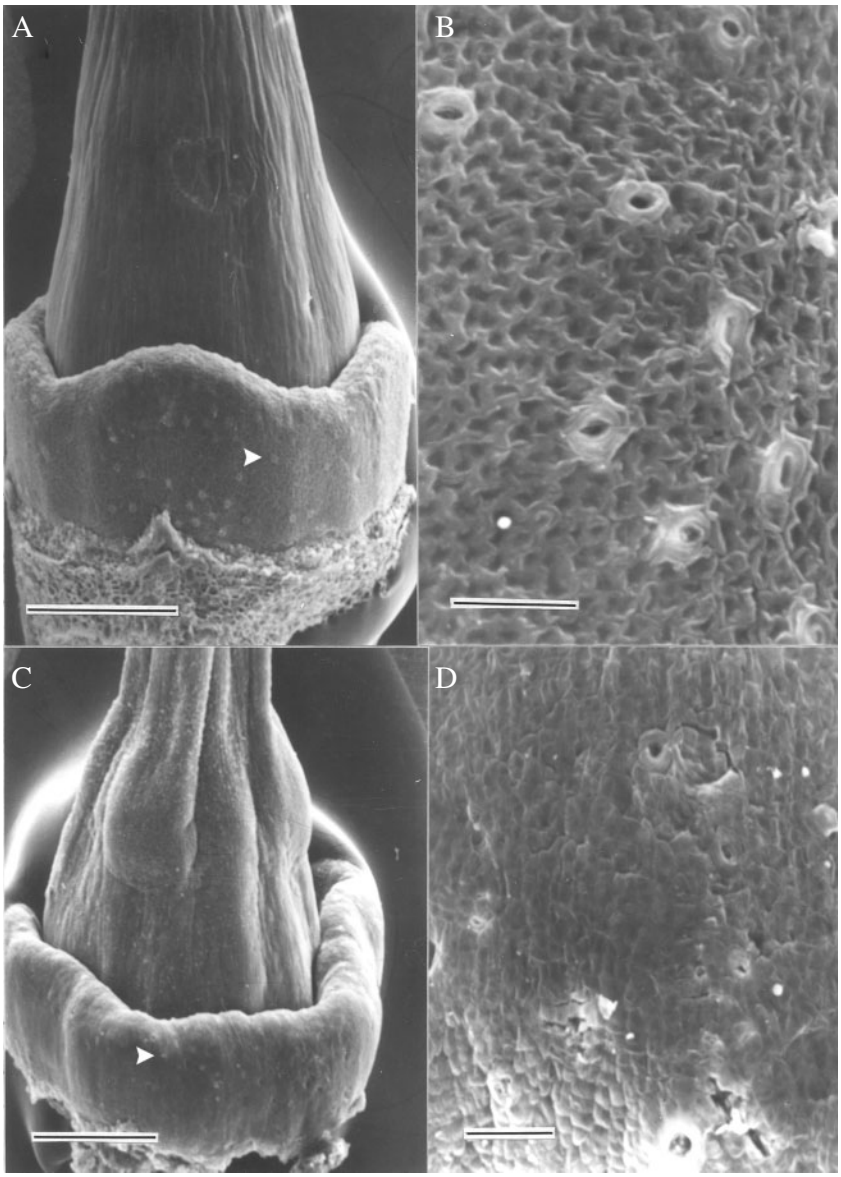

FIG. 1. Nectary SEM photomicrographs: (A and B) *Ipomoea rubriflora*, (C and D) *I. purpurea*. (A and C) View of ovary with the nectary surrounding its base; arrow head points to one stoma. (B and D) Detail of the nectary epidermis showing several stomata. Scale bars: A and C = 500 μm; B and D = 50 μm.

Regressions between flower traits and total nectar volume

Ipomoea alba with longer flower tubes correspondingly had the highest mean total nectar volume per flower and nectary volume (Fig. 3). In the remaining taxa, total volume ranged from $\bar{x}$ = 2·7 μL in *I. purpurea* to $\bar{x}$ = 12·0 μL in *I. hieronymi*, whereas nectary size ranged from $\bar{x}$ = 0·6 mm^3 ± 0·3 in *I. rubriflora* to $\bar{x}$ = 6·9 mm^3 ± 1·1 in *I. hieronymi* (Fig. 3).

Significant positive regressions were found indicating an increase among three parameters: the longer the flower, the more voluminous the nectary and the higher the nectar volume secreted ($R^2 = 0{\cdot}92$, $P = 0{\cdot}02$, $R^2 = 0{\cdot}99$, $P < 0{\cdot}0001$, respectively; Fig. 3). The number of stomata was not significantly correlated with nectary size and nectar volume (R^2 = 0·25, P = 0·65; R^2 = 0·45, P = 0·44, respectively).

DISCUSSION

The morphology and location of the nectaries found in the *Ipomoea* species studied follow the general pattern known for other representatives of the genus (e.g. Fahn, 1979; Stucky and Beckmann, 1982; Pinheiro and Schlindwein, 1998) and seems to be a conservative character for the family (Cronquist, 1981).

Studing nectar may help to determine taxonomic affinities of the species concerned and on the adaptation to the pollinators that visit the taxa. The six *Ipomoea* species analysed included a wide range of floral colours and visitors,

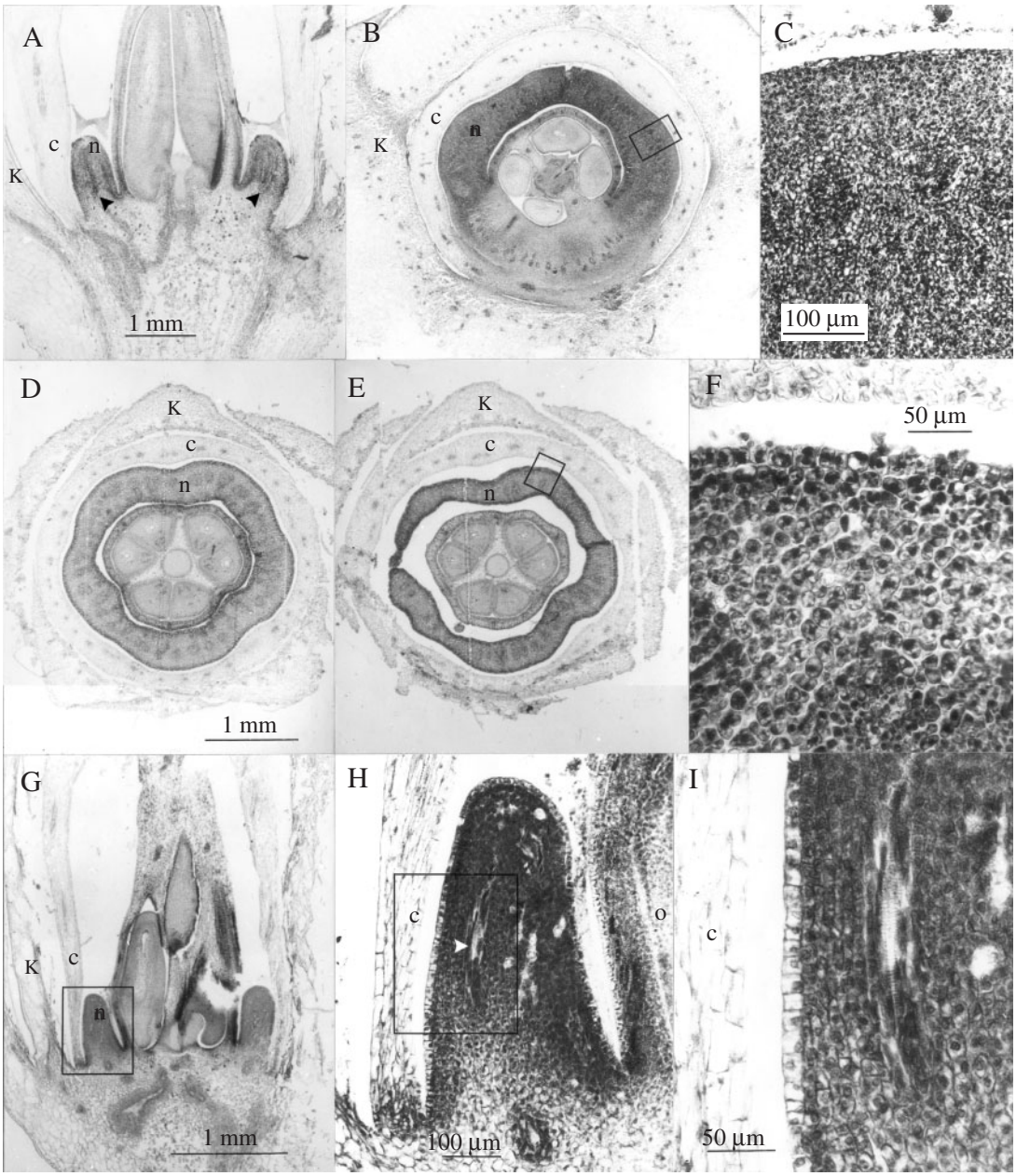

FIG. 2. Optical microscope photomicrographs showing nectary structure: (A–C) *Ipomoea hieronymi*; (D–F) *I. indica*; (G–I) *I. rubriflora*. (A) Flower partial longitudinal section; (B) flower cross-section; (C) detail of the nectariferous tissue indicated in B; (D–E) flower cross-sections at lower and upper levels of the nectary; (F) detail of the nectariferous tissue outlined in E; (G) flower partial longitudinal section; (H) detail of nectary outlined in G; (I) detail of the nectariferous tissue indicated in H. Abbreviations: k, calyx, c, corolla, n, nectary, o, ovary. Arrow heads indicate vascular bundles. A and B at the same scale; D and E at the same scale.

a common feature for the whole genus (e.g. van der Pijl, 1954; Vogel, 1954; Schlising, 1970; Sobreira-Machado and Sazima, 1987; McDonald, 1991). Accordingly, differences in nectar features are to be expected, as found here. However, the differences found could not be related to the pollinator guild of the plants; only the hawk-moth-pollinated *I. alba* is typical for having higher nectar volume, as previously reported (e.g. Cruden *et al*., 1983; Opler, 1983). Thus, generalizations for nectar traits and pollinator relationships are precluded in these *Ipomoea* species.

Nectar secretion can be evaluated with regard to volume or milligrams of sugar, or both. Some authors have studied the effect of nectar removal only considering volume data and found that plants modify secretion as a function of the removals (e.g. Zimmerman and Pyke, 1986). Volume data are not enough to characterize flower costs of nectar secretion (nectar sugar production is more costly to the plant compared with water). Thus, if the sugar production is not known it is impossible to evaluate both the costs of secretion and the energetic reward value for the pollinators.

TABLE 3. *Chemical composition of nectar in* Ipomoea *species*

Species	Population no.	Sugars (%)			*r*	*hr*	*hs*
		Sucrose	Fructose	Glucose			
I. alba	1	87·19 ± 4·84	8·78 ± 3·21	4·01 ± 2·53	6·81	0·45	5
	2	63·14 ± 2·01	20·43 ± 1·84	16·42 ± 2·41	1·71	0·80	5
	3	82·21 ± 3·88	13·80 ± 4·24	3·98 ± 0·97	4·62	0·28	4
Mean		77·51 ± 12·7	14·33 ± 5·84	8·13 ± 7·17	3·45	0·56	
I. cairica	2	0·52 ± 0·37	34·54 ± 2·06	64·93 ± 2·42	0·005	1·87	5
	5	35·62 ± 3·34	18·81 ± 1·72	45·52 ± 2·09	0·55	2·41	6
	3	0·37 ± 0·30	36·52 ± 3·69	63·10 ± 3·99	0·003	1·72	2
	1	0·34 ± 0·23	32·33 ± 1·68	67·32 ± 2·87	0·003	2·08	5
	4	15·43 ± 2·12	36·86 ± 1·38	47·68 ± 0·75	0·18	1·29	4
Mean		10·45 ± 15·5	31·81 ± 7·49	57·71 ± 10·3	0·12	1·81	
I. indica	4	55·74 ± 4·05	18·41 ± 3·92	25·84 ± 2·13	1·25	1·40	6
	3	50·74 ± 6·71	21·12 ± 1·81	28·12 ± 4·90	1·03	1·33	7
	2	54·31 ± 1·89	18·81 ± 2·13	26·87 ± 1·37	1·18	1·42	7
Mean		53·60 ± 2·58	19·45 ± 1·46	26·94 ± 1·14	1·15	1·39	
I. hieronymi	2	60·39 ± 10·97	15·58 ± 6·22	24·01 ± 5·25	1·52	1·54	2
	1	65·04 ± 5·76	11·03 ± 2·49	23·91 ± 3·27	1·86	2·17	2
Mean		62·72 ± 3·28	13·31 ± 3·21	23·96 ± 0·07	1·68	1·80	
I. rubriflora	2	66·08 ± 4·98	22·13 ± 5·00	11·78 ± 2·52	1·94	0·53	6
	1	64·86 ± 4·57	19·17 ± 3·88	15·96 ± 2·61	1·84	0·83	6
	3	62·61 ± 8·93	13·95 ± 8·74	23·42 ± 4·33	1·67	1·67	5
Mean		64·52 ± 1·76	18·42 ± 4·14	17·05 ± 5·89	1·82	0·93	
I. purpurea	4	68·71 ± 5·07	7·48 ± 2·55	23·79 ± 2·50	2·19	3·18	3
	2	65·74 ± 7·52	14·01 ± 1·67	20·24 ± 3·84	1·91	1·44	3
	3	69·63 ± 6·62	8·96 ± 4·00	21·41 ± 2·93	2·29	2·38	5
	1	78·68 ± 2·00	10·27 ± 0·85	11·04 ± 1·14	3·69	1·07	5
	5	60·08 ± 5·51	15·88 ± 3·37	24·03 ± 4·07	1·50	1·51	4
Mean		68·57 ± 6·77	11·32 ± 3·52	20·10 ± 5·31	2·18	1·78	

Values are means ± s.d.
r, Sugar ratio; *hr*, hexose ratio; *hs*, histidine scale.
Population nos corresponds to those in Table 1 and the number of individuals sampled for each one was four.

Concerning the nectar sugar composition, only *I. cairica* had hexose-predominant nectar, a composition preferred by short-tongued bees (Baker and Baker, 1983*a*). Regardless of the pollinator, almost all species had a sucrose-dominant nectar. This is an unusual result for bee-pollinated species, which commonly have hexose predominant nectars (Baker and Baker, 1983*a*; Galetto and Bernardello, 2003). Hummingbird-pollinated flowers tend to have sucrose-dominant nectar (e.g. Baker and Baker, 1983*a*; Freeman *et al*., 1985; Stiles and Freeman, 1993), which is confirmed for *I. rubriflora* among the climbers studied. Thus, no generalization regarding sugar composition and pollinator preference can be shown.

Several authors have found that taxonomically related plants showed a similar trend in their nectar sugar composition because they share common ancestors, rather than because they share the same floral visitors (e.g. Elisens and Freeman, 1988; van Wyk, 1993; Galetto *et al*. 1998; Perret *et al*., 2001; Torres and Galetto, 2002). In *Ipomoea*, two extreme trends related to nectar sugar composition were observed, hexose or sucrose predominant, but they cannot be related to the pollinators or to phylogenetic constraints; however, considering the high number of species in the genus and the scarcity of taxa studied, more data are needed to understand the significance of these results.

Differences were also found among the *Ipomoea* species studied here in terms of nectar production dynamics. Most of them secreted nectar continuously during the whole lifetime of the flower, whereas *I. purpurea* and *I. rubriflora* (the species that has the smallest quantity of nectar per flower) secreted most of it during the first hour of the flower lifetime. Previous data on a few other *Ipomoea* species, although scant, agree on the whole with the findings reported here (Real, 1981; Stucky and Beckmann, 1982; Devall and Thien, 1989). In particular, *I. batatas* (Real, 1981) showed similar production dynamics to *I. hieronymi* studied here. In contrast, in *I. pandurata* (Stucky and Beckmann, 1982) nectar began to be secreted in the bud and had a comparatively large total volume for a bee-pollinated plant (cf. Opler, 1983). Nevertheless, nectar production dynamics and removal effects, together with sugar composition, could not be clearly related either to the pollinator guild or the breeding system of the species involved. The SI species are all bee-pollinated (*I. hieronymi*, *I. indica*, *I. cairica*) and showed a similar total nectar production, but had differences in their nectar composition (*I. hieronymi* and *I. indica* are sucrose-dominant, whereas *I. cairica* is hexose-dominant). The SC species (*I. alba*, *I. purpurea*, *I. rubriflora*) showed no variation in nectar composition (sucrose-dominant nectars), but significant differences in their nectar production pattern.

In contrast, flower length was associated with both nectary size and total amount of nectar produced. Recent studies suggest that flower morphology is evolutionarily more labile and that corolla traits can frequently change (e.g. Cubas *et al*., 1999; Harrison *et al*., 1999) in comparison

TABLE 4. *Nectar sugar concentration (% of sucrose, wt/wt), nectar volume (μL), and mg of sugar of six Argentinian Ipomoea species measured in flower sets subjected to different removal schedules throughout the lifetime of the flower*

(a) *I. alba* (each flower set $n = 10$)

	Time of sampling (hours after flower opening)					
	2000 (1)	2300 (4)	0200 (7)	0500 (10)	0800 (13)	Total amount of sugar per flower (mg)
Set 1						
Conc.	35·14 ± 1·63	6·42 ± 2·76	19·00 ± 3·26	14·42 ± 1·81	13·50 ± 3·90	17·31 ± 5·65
Volume	10·28 ± 1·57	31·14 ± 16·0	9·85 ± 3·23	11·85 ± 13·7	13·50 ± 3·90	
mg sugar	3·98 ± 0·58	9·22 ± 4·74	1·98 ± 0·67	1·81 ± 2·22	0·32 ± 0·29	
Set 2						
Conc.		36·14 ± 0·75	28·07 ± 1·30	21·21 ± 1·82	17·14 ± 2·26	21·06 ± 4·94
Volume		31·42 ± 0·69	17·42 ± 9·10	4·64 ± 1·18	8·71 ± 10·87	
mg sugar		13·06 ± 5·30	5·49 ± 2·79	1·09 ± 0·35	1·41 ± 1·86	
Set 3						
Conc.			34·42 ± 1·94	27·85 ± 2·54	23·07 ± 2·49	22·15 ± 7·80
Volume			46·28 ± 1·90	11·42 ± 13·9	3·14 ± 0·89	
mg sugar			17·88 ± 5·07	3·45 ± 3·96	0·81 ± 0·24	
Set 4						
Conc.				34·14 ± 1·87	25·35 ± 2·71	17·69 ± 3·76
Volume				43·71 ± 1·75	3·00 ± 0·57	
mg sugar				16·84 ± 3·87	0·84 ± 0·19	
Set 5 (control)						
Conc.					31·06 ± 2·02	19·41 ± 4·30
Volume					50·12 ± 2·02	
mg sugar					19·41 ± 4·30	

(b) *I. cairica* (each flower set $n = 7$)

	Time of sampling (hours after flower opening)				
	0930 (2)	1230 (5)	1430 (7)	1700 (9·5)	Total amount of sugar per flower (mg)
Set 1					
Conc.	52·10 ± 5·64	46·70 ± 3·77	28·44 ± 2·60	29·33 ± 4·45	5·05 ± 0·49[a]
Volume	1·55 ± 0·43	4·70 ± 0·73	3·25 ± 1·71	0·90 ± 0·99	
mg sugar	1·00 ± 0·32	2·63 ± 0·32	1·14 ± 0·39	0·29 ± 0·29	
Set 2					
Conc.		52·60 ± 5·56	35·00 ± 5·92	30·87 ± 4·01	5·46 ± 0·75[a]
Volume		4·20 ± 0·78	5·40 ± 1·50	1·75 ± 1·11	
mg sugar		2·72 ± 0·46	2·14 ± 0·29	0·61 ± 0·39	
Set 3					
Conc.			47·60 ± 5·75	36·80 ± 3·85	3·84 ± 0·65[b]
Volume			5·10 ± 1·97	2·30 ± 0·92	
mg sugar			2·89 ± 1·04	0·96 ± 0·35	
Set 4 (control)					
Conc.				44·70 ± 5·39	4·18 ± 1·09[b]
Volume				8·00 ± 5·39	
mg sugar				4·18 ± 1·09	

(c) *I. hieronymi* var. *hieronymi* (each flower set $n = 7$)

	Time of sampling (hours after flower opening)				
	0800 (1)	1030 (3·5)	1300 (6)	1530 (8·5)	Total amount of sugar per flower (mg)
Set 1					
Conc.	34·42 ± 4·45	26·31 ± 6·15	29·21 ± 4·22	22·44 ± 3·59	3·13 ± 0·25[a]
Volume	1·35 ± 0·41	1·75 ± 0·74	2·81 ± 1·37	5·20 ± 1·52	
mg sugar	0·55 ± 0·24	0·50 ± 0·43	0·89 ± 0·28	1·19 ± 0·37	
Set 2					
Conc.		38·14 ± 1·76	31·00 ± 4·42	28·72 ± 6·66	3·00 ± 0·41[a]
Volume		2·28 ± 0·67	2·14 ± 1·09	4·07 ± 0·92	
mg sugar		1·01 ± 0·30	0·69 ± 0·22	1·30 ± 0·45	
Set 3					
Conc.			37·66 ± 3·07	29·55 ± 4·82	2·53 ± 0·61[a]
Volume			2·70 ± 0·75	4·00 ± 1·61	
mg sugar			1·19 ± 0·37	1·33 ± 0·40	
Set 4 (control)					
Conc.				36·64 ± 4·04	5·01 ± 2·11[b]
Volume				12·00 ± 5·21	
mg sugar				5·01 ± 2·11	

(d) *I. indica* (each flower set $n = 10$)

	Time of sampling (hours after flower opening)					
	0800 (1)	1100 (4)	1400 (7)	1700 (10)	2000 (13)	Total amount of sugar per flower (mg)
Set 1						
Conc.	34·83 ± 2·22	34·83 ± 2·22	36·33 ± 1·86	31·5 ± 2·51	23·25 ± 4·03	5·47 ± 1·19[a]
Volume	3·00 ± 0·63	4·33 ± 0·2	3·33 ± 1·03	2·41 ± 2·06	0·75 ± 0·75	
mg sugar	1·21 ± 0·30	1·73 ± 0·34	1·44 ± 0·47	0·89 ± 0·79	0·20 ± 0·23	
Set 2						
Conc.		38·50 ± 1·51	8·16 ± 2·99	2·33 ± 2·42	8·50 ± 1·22	6·07 ± 0·74[a]
Volume		5·66 ± 1·51	3·33 ± 0·81	3·75 ± 0·75	2·00 ± 1·09	
mg sugar		2·55 ± 0·35	1·48 ± 0·35	1·40 ± 0·40	0·64 ± 0·37	
Set 3						
Conc.			39·83 ± 2·04	35·16 ± 2·13	3·66 ± 4·80	5·75 ± 0·79[a]
Volume			6·66 ± 0·81	3·66 ± 0·51	4·33 ± 3·14	
mg sugar			3·11 ± 0·44	1·50 ± 0·22	1·13 ± 0·79	
Set 4						
Conc.				40·00 ± 1·41	33·50 ± 2·81	4·77 ± 0·85[ab]
Volume				8·00 ± 1·55	2·58 ± 0·49	
mg sugar				3·76 ± 0·70	1·01 ± 0·22	
Set 5 (control)						
Conc.					36·83 ± 1·72	3·98 ± 0·69[b]
Volume					9·33 ± 1·63	
mg sugar					3·98 ± 0·69	

(e) *I. purpurea* (each flower set $n = 15$)

	Time of sampling (hours after flower opening)				
	0830 (1)	1130 (4)	1430 (7)	1730 (10)	Total amount of sugar per flower (mg)
Set 1					
Conc.	38·00 ± 4·17	25·30 ± 5·50	–	–	1·50 ± 0·23[a]
Volume	2·40 ± 0·45	1·30 ± 0·80	0	0	
mg sugar	1·05 ± 0·15	0·45 ± 0·35	0	0	
Set 2					
Conc.		40·00 ± 1·70	23·20 ± 1·6	–	1·55 ± 0·62[a]
Volume		3·10 ± 0·76	0·46 ± 0·32	0	
mg sugar		1·35 ± 0·51	0·20 ± 0·21	0	
Set 3					
Conc.			41·50 ± 6·37	–	1·32 ± 0·28[a]
Volume			2·85 ± 0·83	0	
mg sugar			1·32 ± 0·28	0	
Set 4 (control)					
Conc.				38·20 ± 7·59	1·24 ± 0·12[b]
Volume				2·42 ± 0·82	
mg sugar				1·24 ± 0·12	

(f) *I. rubriflora* (each flower set $n = 20$)

	Time of sampling (hours after flower opening)				
	0830 (1·5)	1130 (4·5)	1330 (6·5)	1530 (8·5)	Total amount of sugar per flower (mg)
Set 1					
Conc.	25·15 ± 2·20	19·72 ± 5·51	–	–	0·80 ± 0·39
Volume	2·43 ± 0·88	0·64 ± 0·02	0	0	
mg sugar	0·67 ± 0·27	0·13 ± 0·16	0	0	
Set 2					
Conc.		24·98 ± 2·42	–	–	0·90 ± 0·33
Volume		3·16 ± 0·99	0	0	
mg sugar		0·90 ± 0·33	0	0	
Set 3					
Conc.			25·17 ± 2·50	–	0·88 ± 0·33
Volume			3·11 ± 1·12	0	
mg sugar			0·88 ± 0·33	0	
Set 4 (control)					
Conc.				25·42 ± 2·17	1·03 ± 0·49
Volume				3·73 ± 1·73	
mg sugar				1·03 ± 0·49	

Data on the diagonal (first measurements of each set underlined) correspond to the nectar production dynamics of each species.
The total amount of sugar per flower was calculated for each set using set 4 or 5 as control, according to the species. When statistically significant differences were found, lower-case letters as superscript indicate *a posteriori* test results.

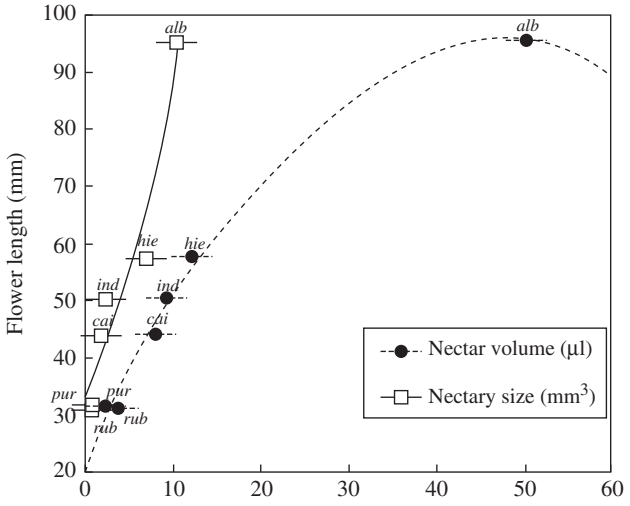

FIG. 3. Quadratic regressions between flower length and nectar volume and nectary size in six Argentinian species of *Ipomoea*. Plotted values represent the arithmetic mean of each species (abbreviated species' names are shown).

to changes in the nectar features (e.g. van Wyk, 1993; Galetto *et al*., 1998; Perret *et al*., 2001; Torres and Galetto, 2002). The association found here between flower size and total nectar volume secreted in *Ipomoea* suggests that structural constraints may play a major role in conserving nectar traits, at least in volume.

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