



Nectar Sugar Composition in Relation to Pollination Syndromes in Sinningieae (Gesneriaceae)

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A putative correlation between nectar sugar composition and pollination syndrome was evaluated in the tribe Sinningieae (Neotropical Gesneriaceae). Sucrose, fructose and glucose were quantified in the nectar of 45 species using high performance anion-exchange chromatography. Representative species of the hummingbird, bee, bat and moth pollination syndromes were sampled in relation to their numeric importance in the tribe. In hummingbird and bee flowers, which represent 95% of the species in Sinningieae, nectar was sucrose-dominant (ratio [sucrose]/[hexose] > 1). Sugar ratios below one were only found in the nectar of three species with moth and bat syndromes. Sugar concentration averaged $23.9 \pm 10.6\%$ (wt/total wt) in hummingbird flowers and $28.7 \pm 10.6\%$ in bee flowers, whereas diluted nectar ($7.1 \pm 3.4\%$) was restricted to bat flowers. Similarities in the nectar of hummingbird and bee flowers contrast with the presence of specific morphological traits associated with these two syndromes, indicating that plant-pollinator relationships rely on flower display rather than on nectar characteristics. By contrast, distinct nectar chemistry is correlated with the bat syndrome in which a particularly low sucrose production is responsible for hexose dominance.

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INTRODUCTION

Nectar plays a key role in plant reproduction by rewarding pollinators (Simpson and Neff, 1983). Its accessibility in relation to floral morphology, but also its inherent features such as concentration, volume, viscosity and chemical composition, determines plant-pollinator relationships (Heinrich and Raven, 1972; Baker and Baker, 1983a; Kingslover and Daniel, 1983; Proctor *et al.*, 1996). Nectar is mainly composed of three sugars: two hexoses (glucose and fructose) and their combination into a disaccharide, sucrose, amounts and relative concentrations of which vary among species (Percival, 1965). Proportions of sucrose over fructose and glucose have been linked with different classes of pollinators. Sucrose-rich nectar has been found mostly in flowers pollinated by hummingbirds or insects with long mouth parts (*viz.* long-tongued bees, moths and butterflies), whereas hexose-rich nectar has been found in flowers pollinated by short-tongued bees, bats, perching birds and flies (see Baker and Baker, 1983b; Freeman *et al.*, 1984; Lammers and Freeman, 1986; Elisens and Freeman, 1988; Baker and Baker, 1990; Stiles and Freeman, 1993; Baker *et al.*, 1998). Because associations between nectar features and pollination biology have occurred independently in different angiosperm lineages, several authors have suggested that this convergence is the result of plant

adaptation to preferences, digestive abilities or sugar intake efficiency of specific pollinators (e.g. see Heyneman, 1983; Martinez del Rio *et al.*, 1992; Baker *et al.*, 1998).

Most studies on nectar chemistry have been designed to reveal convergence among ecological or geographical plant communities, but few concern nectar comparisons between species belonging to the same genus or tribe. However, an analysis in Anthirrhineae and *Lycium* has revealed constant sugar composition despite a large variety of pollinators (Elisens and Freeman, 1988; Galetto *et al.*, 1998). In the genus *Erythrina*, contrasting sucrose proportions were correlated to pollination by either hummingbirds or perching birds (Baker and Baker, 1983b). To study how nectar chemistry is involved in the adaptation to a specific pollinator spectrum and how phylogenetic constraints act on this chemistry, data on a complete range of closely related species are needed. Here, we document the nectar sugar composition and concentration for 45 species belonging to tribe Sinningieae (Gesneriaceae). Since these species exhibit hummingbird, bee, bat and moth syndromes, this tribe is an ideal one in which to test for correlation between nectar traits and other syndrome characters.

MATERIALS AND METHODS

Taxa investigated

The tribe Sinningieae comprises three genera: *Sinningia* (65 species), *Paliavana* (six species) and *Vanhouttea* (eight

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species) (Chautems, 1990, 1991, 1995; Chautems and Weber, 1999; Chautems *et al.*, 2000; but *Paliavana* and *Vanhouttea* could possibly be merged into the genus *Sinningia*; Perret, Savolainen and Chautems, unpubl. res.) Their habit ranges from herbaceous species with a perennial tuber in *Sinningia* to shrubs in *Paliavana* and *Vanhouttea*; they are predominantly saxicolous or epiphytic, occasionally terrestrial (Chautems and Weber, 1999). Species are distributed throughout the Neotropics, with the highest number of species occurring in southeastern Brazil; they occur mostly on rocky outcrops within the Atlantic rain forest between sea level and 2000 m in altitude.

Floral morphology is typical for Neotropical Gesneriaceae with gamopetalous, mostly zygomorphic corollas with four protandrous and nototribic coherent anthers. Nectar is produced by glands around the ovary and accumulates at the base of corolla-tubes in two or five gibbositities. In this study, the notion of pollination syndrome was used as a tool to predict the pollinator spectrum. This approach has been criticized by Herrera (1996) and Waser *et al.* (1996), who advocated more generalist and dynamic interactions in pollination systems than the traditional concept of pollination syndrome. However, pollination syndromes have proved accurate in predicting hummingbird, bat and hawkmoth pollinators in most New World species (Cruden, 1997). Thus, putative pollinators (*viz.* hummingbirds, bats, bees and moths, see Fig. 1A–F), were inferred from a set of floral characters, including corolla shape, colour, presence of nectar guides and scent following the definition of these syndromes (see Vogel, 1969; Silberbauer-Gottsberger and Gottsberger, 1975; Faegri and van der Pijl, 1980; Helversen, 1993; Endress, 1994; Proctor *et al.*, 1996). Effective visitors were also confirmed by fieldwork for the hummingbird syndrome. Sazima *et al.* (1996) observed that two hummingbird species, *Leucochloris albicollis* and the hermit *Phaethornis eurynome*, were the main visitors to *Sinningia douglasii* in the mountain forest of Campos do Jordão (state of São Paulo). *Phaethornis ruber* was observed visiting *S. nordestina* in northeastern Brazil (Chautems *et al.*, 2000). Snow and Teixeira (1982) mentioned that *S. cooperi* was one of the main nectar sources for *P. eurynome* in the rainforest of Serra dos Orgãos (state of Rio de Janeiro).

Nectar sampling

Nectar was sampled in 45 species (one to three individuals per species; total 117 samples, see Table 1) among the 80 known species in *Sinningieae*: hummingbird flowers (33 species), bee flowers (nine species), bat flowers (two species out of a total of three species with this syndrome) and a moth flower (the only species with this syndrome). Nectar was collected mainly from plants grown in tropical glasshouses in Geneva, Switzerland and in São Paulo, Brazil. For a few species, nectar from both cultivated and wild plants was obtained. Nectar samples were extracted with a micropipette from a single flower or from a pool of flowers (see Table 1) and spotted on Whatmann filter paper. For each flower, volume of nectar was recorded. Samples were then dried using silica gel and

kept at -20°C until analysis. Nectar was collected at the end of the male phase; therefore, volume measurements correspond to nectar accumulation during a period of 24 to 48 h after the beginning of anthesis. In the glasshouses, nectar amount reflects only the secretion process, whereas nectar amount taken from unbagged flowers growing outside reflects both secretion and depletion by visitors.

Nectar analysis

Separation and quantification of sugars were carried out using high performance anion-exchange chromatography (HPAEC) in the Laboratory of Pharmaceutical Analytical Chemistry (University of Geneva, Switzerland). Data were generated on a Dionex DX 500 chromatography system (Sunnyvale, CA, USA) equipped with a GP50 gradient pump and an ED40 Electrochemical detector. A Dionex cell fitted with a gold working-electrode was used for all experiments. Injections were made using a Waters 717 plus autosampler (Milford, MA, USA). The HPAEC CarboPac MA1 column (250 mm \times 4 mm I.D.) with associated guard was supplied by Dionex. Sodium hydroxide (700 mM) was used as mobile phase at a flow rate of 0.4 ml min^{-1} and standard waveform was used following the manufacturer's protocols ($E1 = 0.05\text{ V}$, $t1 = 400\text{ ms}$, $E2 = 0.75\text{ V}$, $t2 = 200\text{ ms}$, $E3 = -0.15\text{ V}$, $t3 = 400\text{ ms}$). Standard solutions of glucose, fructose and sucrose were prepared in distilled water; calibration curves were calculated in the range $5\text{--}100\text{ mg ml}^{-1}$. Dried sugars were dissolved in 1–5 ml of water by sonication of the filter papers for 15 min. After filtration, only very concentrated samples were further diluted to reach the calibration domain. Chromatographic sugar analyses were made three times for each sample. Sugar ratio was calculated as $[\text{sucrose}]/[\text{fructose}] + [\text{glucose}]$. Concentrations were converted from g l^{-1} to sucrose-equivalent, % weight per total weight, using Table 63 in the 50th edition of the Handbook of Chemistry and Physics (Weast, 1969). Because data were not normally distributed, Wilcoxon rank-sum tests were used for non-parametric comparison of species means among the classes of pollination syndromes.

RESULTS

Nectar sugar concentration and composition are presented in Table 1. Nectars were sucrose-dominant in all hummingbird and bee flowers, with the proportion of sucrose ranging from 57.8 to 88.6%. In these species, the sucrose/hexose ratios were always higher than one, a value that is typical of hummingbird and large bee flowers according to Baker and Baker (1990). Within these two types of flowers, the mean sucrose/hexose ratio was higher in hummingbird (4.3 ± 1.4 , $n = 33$) than in bee (3.7 ± 1.5 , $n = 9$) syndromes, but this difference was non-significant ($P > 0.05$). Sucrose/hexose ratios below one were only found in three species—*S. tubiflora* (moth syndrome), *S. brasiliensis* and *P. prasinata* (both with bat syndrome, Table 1). With a ratio of 0.2, *P. prasinata* is the only species with a hexose-rich nectar (ratio < 0.5 , see Baker and Baker, 1990). Comparison of Figs 2 and 3 shows that differences in sugar

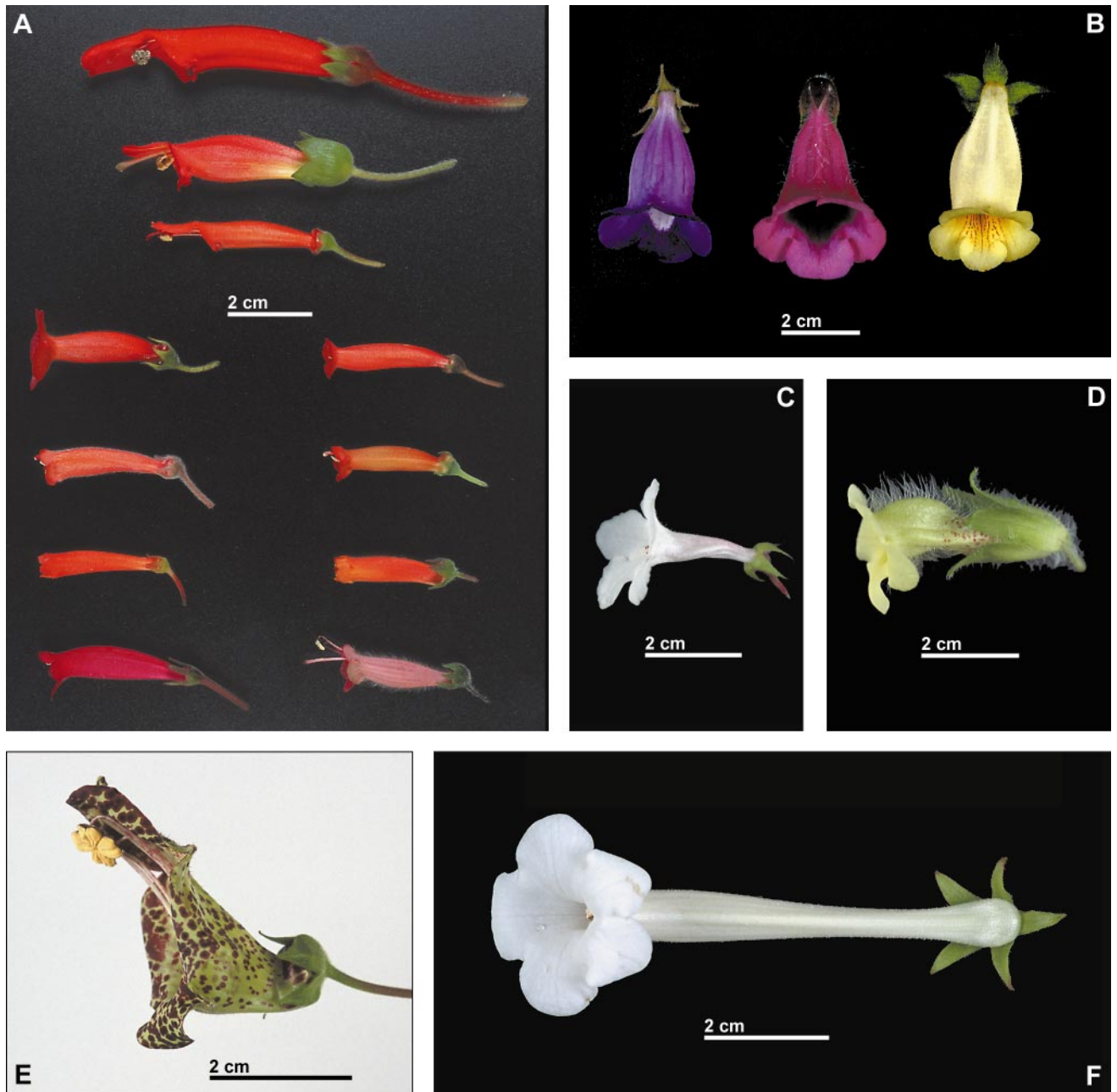


FIG. 1. Pollination syndromes in *Sinningia*. A, Hummingbird flowers have pink, orange or red corollas and present two morphological types: (1) tubular flowers with a limb composed of five equal radially symmetric lobes (bottom, left and right rows *S. lineata*, *S. leucotricha*, *S. insularis*, *S. reitzii*, *S. macropoda*, *S. macrostachya*, *S. aggregata*, *S. selowii*); and (2) long-tubular flowers with an upper horizontal lip and three small lobes in ventral and lateral positions (from top to bottom *S. cardinalis*, *S. incarnata*, *S. bulbosa*). B, Bee flowers with bell-shape corollas 4–5 cm in length (from left to right *S. speciosa*, *S. aghensis*, *S. conspicua*). C and D, Bee flowers with narrow straight tubes (C, *S. schiffneri*; D, *S. villosa*). E, Bat flower of *S. brasiliensis* with a corolla that is gullet-shaped, yellow-green, covered with dark spots and producing a scent reminiscent of burnt plastic. F, Moth flower of *S. tubiflora* with a long, narrow, tubular white corolla (6–7 cm) producing a strong sweet fragrance.

proportions were mainly due to variation in sucrose rather than hexose concentration. For example, sucrose concentrations averaged 215 g l^{-1} in species with hummingbird syndrome and fell to a mean of 17 g l^{-1} in those with bat syndrome, but in both cases hexose concentration was approx. 50 g l^{-1} (Fig. 3). Low sucrose/hexose ratios such as those found in *S. brasiliensis* and *P. prasinata* are therefore a consequence of a particularly low production of sucrose.

Considering hexoses only, the proportion of fructose was always higher than that of glucose, except in *P. prasinata* (Table 1). The fructose/glucose ratio was particularly unbalanced in hummingbird, bee and moth flowers since the proportion of glucose was low ($2.7 \pm 3.4\%$) in comparison with fructose ($19.3 \pm 6.9\%$). In eight species with hummingbird and bee flowers, no glucose was detected (Table 1). In contrast, the glucose proportion of

TABLE 1. Sugar concentration and composition (mean \pm s.d.) in nectar of 45 species within *Sinningia*, *Paliavana* and *Vanhouttea* classified according to their pollination syndrome

Species	<i>n</i>	Nf (Ni)	Conc. (%wt/ total wt)	% Sucrose	% Glucose	% Fructose	S/H
Sphingophilous syndrome							
<i>S. tubiflora</i> (Hook.) Fritsch	1	1 (1)	20.5	41.4	8.5	50.1	0.7
Chiropterophilous syndrome							
<i>P. prasinata</i> (Ker-Gawler) Benth.	4	5 (2)	7.6 \pm 2.8	15.5 \pm 0.3	48.9 \pm 0.8	44.7 \pm 0.7	0.2 \pm 0.0
<i>S. brasiliensis</i> (Regel & Schmidt) Wiehler & Chautems	3	6 (3)	6.5 \pm 3.5	37.7 \pm 6.9	27.5 \pm 3.6	34.7 \pm 3.7	0.6 \pm 0.2
Mellitophilous syndrome							
<i>S. aghensis</i> Chautems	3	12 (3)	18.3 \pm 3.6	77.1 \pm 4.8	0.3 \pm 0.4	22.6 \pm 5.1	3.6 \pm 0.9
<i>S. barbata</i> (Nees & Mart.) G. Nicholson	2	4 (2)	7.0–45.6	71.9–77.4	0–0.4	22.3–28.1	2.6
<i>S. conspicua</i> (Seem.) G. Nicholson	1	2 (1)	32.5	82.4	0.1	17.5	4.7
<i>S. eumorpha</i> H. E. Moore	3	3 (1)	31.1 \pm 2.9	72.3 \pm 13.7	12.3 \pm 6.7	15.4 \pm 7.1	3.4 \pm 1.7
<i>S. lindleyi</i> Schauer	4	4 (2)	38.1 \pm 2.4	61.4 \pm 9.2	6.9 \pm 3.7	31.7 \pm 5.9	1.8 \pm 0.7
<i>S. schiffneri</i> Fritsch	4	7 (3)	38.6 \pm 4.4	79.1 \pm 5.3	7.3 \pm 1.5	13.7 \pm 4.5	4.1 \pm 1.3
<i>S. speciosa</i> (Lodd.) Hiern	6	7 (4)	27.3 \pm 10.0	76.6 \pm 8.7	3.4 \pm 3.7	19.9 \pm 5.0	4.2 \pm 1.6
<i>S. villosa</i> Lindl.	1	1 (1)	34.0	66.3	8.6	25.1	2.0
<i>S. sp. nov. ined. 1</i>	1	4 (1)	8.6	87.1	0	12.9	6.8
Ornithophilous syndrome							
<i>S. aggregata</i> (Ker-Gawler) Wiehler	4	9 (2)	34.5 \pm 13.1	66.9 \pm 9.3	4.4 \pm 4.1	28.7 \pm 5.3	2.3 \pm 0.8
<i>S. allagophylla</i> (Mart.) Wiehler	3	10 (3)	15.2 \pm 6.0	72.8 \pm 10.2	2.1 \pm 2.1	25.1 \pm 8.2	3.3 \pm 1.6
<i>S. bulbosa</i> (Ker-Gawler) Wiehler	6	6 (2)	32.3 \pm 2.6	78.9 \pm 5.0	1.3 \pm 1.3	19.8 \pm 3.9	4.1 \pm 1.4
<i>S. calcaria</i> (Malme) Chautems	2	5 (2)	13.7–16.8	74.4–85.4	0–9.2	14.6–16.4	2.9–5.9
<i>S. canescens</i> (Mart.) Wiehler	3	3 (1)	27.1 \pm 2.2	83.4 \pm 0.6	0.2 \pm 0.3	16.4 \pm 0.4	5.0 \pm 0.2
<i>S. carangolensis</i> Chautems	2	3 (1)	8.7–16.0	63.0–71.2	3.7–4.5	25.1–32.6	1.7–2.5
<i>S. cardinalis</i> (Lehm.) H. E. Moore	4	4 (1)	34.7 \pm 6.0	76.9 \pm 3.4	2.6 \pm 1.3	20.5 \pm 2.9	3.4 \pm 0.6
<i>S. cochlearis</i> (Hook.) Chautems	3	10 (2)	24.5 \pm 7.0	82.0 \pm 1.2	0.5 \pm 0.7	17.5 \pm 1.7	4.6 \pm 0.4
<i>S. cooperi</i> (Paxton) Wiehler	1	5 (1)	8.8	80.0	0	20	4.0
<i>S. douglasii</i> (Lindl.) Chautems	2	5 (2)	10.8–26.1	75.1–76.0	0.6–1.1	22.8–24.3	3.0–3.2
<i>S. elatior</i> (Kunth) Chautems	3	8 (3)	9.7 \pm 5.1	75.1	1.8 \pm 0.6	23.1 \pm 2.4	3.0 \pm 0.3
<i>S. gigantifolia</i> Chautems	1	3 (1)	29.4	82.0	3.5	14.6	4.6
<i>S. hatschbachii</i> Chautems	1	1 (1)	28.9	84.7	0.6	14.7	5.5
<i>S. iarae</i> Chautems	1	1 (1)	33.1	88.6	0	11.4	7.8
<i>S. incarnata</i> (Aubl.) D. L. Denham	3	3 (1)	29.9 \pm 2.4	83.8 \pm 2.4	1.9 \pm 0.6	14.3 \pm 1.8	5.3 \pm 0.9
<i>S. insularis</i> (Hoehne) Chautems	4	4 (1)	33.3 \pm 5.8	82.9 \pm 2.0	2.3 \pm 1.0	14.8 \pm 1.0	4.9 \pm 0.7
<i>S. leucotricha</i> (Hoehne) H. E. Moore	4	4 (1)	29.0 \pm 6.9	84.3 \pm 0.7	1.1 \pm 0.5	14.5 \pm 0.3	5.4 \pm 0.3
<i>S. lineata</i> (Hjelm.) Chautems	6	6 (2)	31.4 \pm 5.4	85.8 \pm 3.0	1.3 \pm 1.5	12.9 \pm 1.9	6.4 \pm 1.6
<i>S. macropoda</i> (Sprague) H. E. Moore	3	5 (2)	19.7 \pm 4.4	75.2 \pm 11.6	2.9 \pm 2.0	21.9 \pm 10.5	3.9 \pm 1.9
<i>S. macrostachya</i> (Lindl.) Chautems	3	3 (1)	28.7 \pm 3.6	83.7 \pm 0.4	0	16.3 \pm 0.4	5.1 \pm 0.2
<i>S. magnifica</i> (Otto & Dietr.) Wiehler	1	3 (1)	8.6	72.7	0	27.3	2.7
<i>S. micans</i> (Fritsch) Chautems	1	3 (1)	12.6	84.4	0	15.6	5.4
<i>S. nivalis</i> Chautems	2	7 (2)	10.6–13.4	75.5–82.2	0–2.2	17.8–22.3	3.1–4.6
<i>S. nordestina</i> Chautems, Baracho & Siqueira Filho.	1	2 (1)	14.2	57.8	2.1	40.1	1.4
<i>S. reitzii</i> (Hoehne) L. E. Skog	5	5 (2)	35.9 \pm 6.4	76.3 \pm 3.6	6.2 \pm 2.3	17.5 \pm 1.3	3.3 \pm 0.7
<i>S. rupicola</i> (Mart.) Wiehler	1	2 (1)	10.4	85.1	0	14.9	5.7
<i>S. sceptrum</i> (Mart.) Wiehler	1	2 (1)	12.3	76.8	2.4	20.8	3.3
<i>S. sellowii</i> (Mart.) Wiehler	3	3 (1)	23.7 \pm 1.9	85.2 \pm 3.1	1.2 \pm 0.9	13.6 \pm 2.3	6.1 \pm 1.6
<i>S. valsuganensis</i> Chautems	3	14 (3)	16.8 \pm 10.2	68.3 \pm 8.4	3.6 \pm 1.9	28.1 \pm 9.9	2.3 \pm 1.0
<i>S. warmingii</i> (Hiern) Chautems	2	7 (2)	5.9–7.6	82.0–82.3	0	17.6–18.0	4.6–4.7
<i>V. sp. nov. ined. 1</i>	2	7 (2)	18.5–23.1	82.0–86.7	1.8–3.4	11.5–14.6	6.5–4.6
<i>V. sp. nov. ined. 2</i>	1	2 (1)	14.6	79.0	8.5	12.6	3.8
<i>V. sp. nov. ined. 3</i>	2	11 (2)	4.5–14.4	82.9–84.9	2.8–3.4	11.7–14.3	4.8–5.6

n, Number of nectar samples analysed. If less than three nectar samples were analysed, effective values are mentioned. Nf, Number of flowers; Ni, number of individuals from which nectar was sampled. S/H = [%sucrose]/[%glucose + %fructose].

nectar in chiropterophilous species was much higher (34.5 \pm 7.1 %).

Total sugar concentrations in nectar were variable among species, especially within bee (28.7 \pm 10.6 %, wt/total wt) and hummingbird flowers (23.9 \pm 10.6 %, wt/total wt), but no significant difference could be found between these two syndromes ($P > 0.05$). With a sugar concentration of 20.5 %, the nectar of moth flowers showed a greater

similarity to the nectar of hummingbird/bee flowers than that of bat flowers, the latter being three–five times more dilute (7.1 \pm 3.4 %). Nectar volume per flower ranged from 2 to 51 μ l in hummingbird flowers (mean \pm s.d. = 18.4 \pm 9.3 μ l), and between 1 and 52 μ l in bee flowers (15.4 \pm 12.1 μ l). The 20 μ l of nectar collected in the moth flower also fell within these ranges (one flower sampled from the only moth flower species). Nectar volumes over 60 μ l

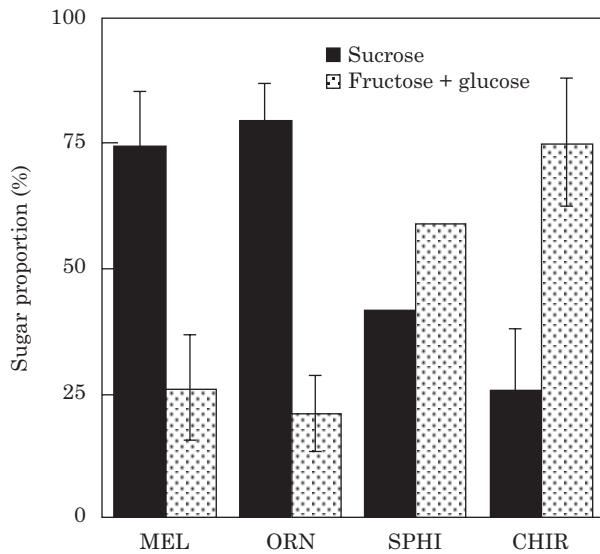


FIG. 2. Mean proportion of sucrose and hexoses (fructose + glucose) in the nectar of 45 *Sinningieae* species arranged according to their pollination syndromes: MEL, melittophilous syndrome ($n = 9$); ORN, ornithophilous syndrome ($n = 33$); SPHI, sphingophilous syndrome ($n = 1$); CHIR, chiropterophilous syndrome ($n = 2$). Vertical bars represent \pm s.d.

(up to 135 μ l; mean = 88.9 μ l) were found only in the two bat flowers (Fig. 4). The large ranges associated with nectar volumes are in part sampling artefacts since we did not take into account the precise time of secretion and depletion by visitors in flowers growing outdoors. Therefore, these values only indicate the range of volumes of nectar that can be found in each syndrome. Nevertheless, these results support the finding that bat-flowers have four–six times the nectar volume of the other species (Fig. 4). Sugar amount in bat flowers is therefore comparable to that in the other *Sinningieae* species since volume and dilution increase by a similar factor.

DISCUSSION

Our results show that sucrose is the dominant constituent in the nectar of all hummingbird and bee flowers among *Sinningieae* (42 out of 45 species analysed). This is in agreement with previous results in other ornithophilous Gesneriaceae (Percival, 1965; Stiles and Freeman, 1993), and in hummingbird flowers from other families (Baker and Baker, 1983b; Freeman *et al.*, 1984; Lammers and Freeman, 1986; Elisens and Freeman, 1988; Stiles and Freeman, 1993; Baker *et al.*, 1998). Similar sucrose-dominant nectars were also found in melittophilous Antirrhineae (Elisens and Freeman, 1988) and Iridaceae pollinated by long-tongued bees (Goldblatt *et al.*, 1998). The low proportion or absence of glucose that characterizes all *Sinningieae* with bee and hummingbird syndromes was also found in Costa Rican ornithophilous Gesneriaceae, Acanthaceae and Musaceae (Stiles and Freeman, 1993). Despite the fact that *S. tubiflora* has a moth syndrome, its sucrose proportion (41.4%) is lower than that reported by Baker and Baker (1983b) for moth flowers (over 50%). On the other hand, the low

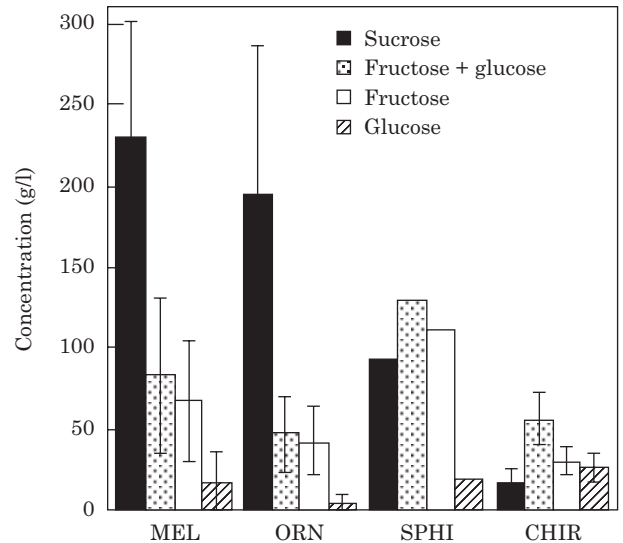


FIG. 3. Sucrose and hexose concentrations in the nectar of 45 *Sinningieae* species arranged according to their pollination syndromes: MEL, melittophilous syndrome ($n = 9$); ORN, ornithophilous syndrome ($n = 33$); SPHI, sphingophilous syndrome ($n = 1$); CHIR, chiropterophilous syndrome ($n = 2$). Vertical bars represent \pm s.d.

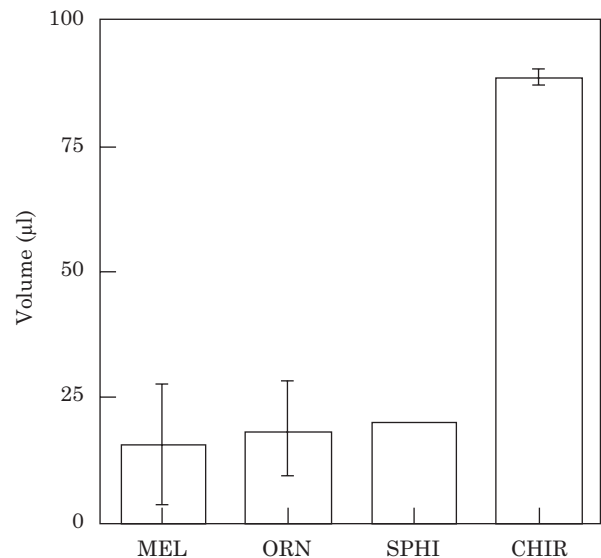


FIG. 4. Nectar volumes per flower collected in the different pollination syndromes: MEL, melittophilous syndrome ($n = 9$); ORN, ornithophilous syndrome ($n = 33$); SPHI, sphingophilous syndrome ($n = 1$); CHIR, chiropterophilous syndrome ($n = 2$). Vertical bars represent \pm s.d.

sucrose proportion found in *P. prasinata* and *S. brasiliensis* is similar to that observed in other Neotropical bat flowers (Baker *et al.*, 1998). These convergences in sugar proportion associated with pollination systems would reflect adaptation to pollinator preferences and, in the case of hummingbirds, feeding experiments have shown a moderate preference for sucrose-rich solutions over those containing fructose and glucose (Hainsworth and Wolf, 1976; Stiles, 1976; Martinez del Rio *et al.*, 1992).

Considering the sugar concentration in nectar of *Sinningieae*, values for hummingbird flowers were consistent with the result of [Stiles and Freeman \(1993\)](#), who found a mean concentration of 23 % (wt/total wt) in 94 hummingbird-pollinated species from Costa Rica, and [Baker \(1975\)](#) and [Pyke and Waser \(1981\)](#), who cited values between 21 and 26 % for various tropical hummingbird-pollinated species. These concentrations were also within the range of the optimal nectar concentration established in feeding experiments with hummingbirds when small volumes were presented (20–25 %, [Kingslover and Daniel, 1983](#); [Tamm and Gass, 1986](#)). Nectar concentrations of bee flowers within *Sinningieae* are generally lower than those recorded in other surveys of bee-pollinated flowers in temperate and tropical regions (25–56 %; [Pyke and Waser, 1981](#)). Dilute nectar observed in the two bat flowers is consistent with measurements from bat-pollinated *Gentianaceae* in northeastern Brazil and an assemblage of bat-pollinated species in two Atlantic forest sites, ([Machado et al., 1998](#); [Sazima et al., 1999](#)). The low sugar concentration characterizing the nectar of bat flowers contrasts with feeding experiments by [Roces et al. \(1993\)](#), who showed that glossophagine bats always preferred the most concentrated solutions, up to a threshold of 50 %. However, [Mitchell and Paton \(1990\)](#) showed that the preferred concentration was much lower when sugar intake was measured using a fixed amount of sugar in a variable volume of water (viz. equal sugar presentation). These results indicate that even dilute nectar could be preferred providing that sufficient sugar was offered. This is the case in the *Sinningieae* since similar amounts of sugar are present even when concentrations and volumes vary ([Figs 3 and 4](#)). To limit a possible reduction of fitness due to over-production of sugar, nectar attracting different pollinators could be produced by diluting an initial constant sugar amount ([Pyke, 1991](#)); in addition, low sugar concentration would deter opportunistic visitors preferring more concentrated rewards ([Bolten and Feinsinger, 1978](#)).

The tribe *Sinningieae* was recently redefined by [Smith and Atkinson \(1998\)](#), who suggested that the genera *Paliavana*, *Vanhouttea* and *Sinningia* were closely related and should be segregated from *Gloxinieae*. A cladistic analysis of *Sinningieae* based on morphological characters ([Boggan, 1991](#)) and a preliminary molecular phylogeny ([Perret et al., 1999](#)) confirmed the monophyly of this tribe and defined several clades in which hummingbird and bee syndromes both occur. Groups identified by [Chautems and Weber \(1999\)](#) based on inflorescence structure, and coenospecies proposed by [Clayberg \(1996\)](#), also include different pollination syndromes. Therefore, floral morphology appears highly labile and contrasts with the great similarity of sugar composition and concentration measured in the bee and hummingbird flowers. Switches among these syndromes have thus relied on characters linked to flower display (flower colour, nectar guides, scent) and to accessibility of the reward (width of the corolla, length of the tube) rather than on nectar characteristics. Nectar chemistry appears to be more conservative than floral morphology, as pointed out by [Galletto et al. \(1998\)](#). In our case, stable sugar chemistry could have promoted the numerous bee/hummingbird shifts according to the selection along the

line of least resistance proposed by [Stebbins \(1974\)](#) and the exaptation of [Gould and Vrba \(1982\)](#). Recent progress in understanding the genetic basis of floral development has shown that changes in corolla features can arise more easily than previously thought, being controlled by a few developmental genes and epigenetic activity ([Armbruster, 1993](#); [Bradshaw et al., 1995](#); [Luo et al., 1996](#); [Cubas et al., 1999](#); [Harrison et al., 1999](#)). Striking changes in nectar composition and concentration were only associated with shifts to bat syndrome. In addition, the two species with this syndrome, *P. prasinata* and *S. brasiliensis*, are not sister species, indicating that bat-flower nectar evolved independently from a concentrated nectar rich in sucrose to one produced by greater water secretion and lower sucrose production ([Boggan, 1991](#); [Perret et al., 1999](#)). In this case, since nectar is easily accessible in bell shaped or gullet-like bat flowers, sugar concentration and composition are as important in determining plant-visitor relationships as is floral morphology. However, a better understanding of nectar chemistry evolution, and its role in plant-pollinator relationships, will benefit from a solid phylogenetic framework and extensive field studies.

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