

Nectar Sugar Composition and Volumes of 47 Species of Gentianales from a Southern Ecuadorian Montane Forest

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- **Background and Aims** This study investigates 47 taxonomically related species (Gentianales), all native to a tropical montane forest in southern Ecuador, in terms of nectar chemistry and nectar volumes in relation to pollination biology.
- **Methods** Nectar volumes of covered (24-h production) and uncovered (standing crop) flowers were measured in the natural habitat. Sucrose, fructose and glucose were quantified in the nectar using high performance liquid chromatography. Flower visitors were observed.
- **Key Results** Nectar sugar concentration did not differ significantly among the pollination syndromes. Regarding sugar composition, the only significant differences were found in chiropterophilous and myiophilous flowers, which had a significantly lower sugar ratio than sphingophilous flowers. A separation of chiropterophilous and myiophilous flowers from the other pollination syndromes is further substantiated by non-linear multidimensional scaling using the chord-normalized expected species shared index of dissimilarity based on nectar sugar compositions. The matrix test revealed no correlation of observed floral visitors to nectar concentrations; however, a weak significant correlation was found between floral visitors and nectar sugar compositions. The nectar volumes of covered and uncovered flowers are related to, and differ significantly among, pollination syndromes. Matrix tests revealed correlation between floral visitors and nectar volume of covered flowers and, to a lesser extent, of uncovered flowers.
- **Conclusions** Sucrose is the predominant floral nectar sugar in the order Gentianales, suggesting that nectar sugar composition is a conservative characteristic. However, some degree of an adaptive convergence of floral nectar compositions to principal pollinator type within the constraints set by phylogenetic history is likely. The driving force to visitation appears to be the volume of nectar the visitor can expect to consume.

Key words: Nectar sugar composition, nectar volume, nectar standing crop, pollination syndrome, Rubiaceae, Gentianaceae, tropical montane forest, Ecuador.

INTRODUCTION

Floral nectar is the most important reward offered to pollinators in angiosperms (Simpson and Neff, 1983). The major sugars in nectar are the disaccharide sucrose and the hexose monosaccharides glucose and fructose (Baker and Baker, 1983). Floral nectar characteristics such as sugar composition, sucrose–hexose proportions, concentration, volume, time of nectar secretion and nectar dynamics are often related to the interaction of flowers and pollinators (Baker and Baker, 1983; Freeman *et al.*, 1984; Baker and Baker, 1990; Stiles and Freeman, 1993; Galetto *et al.*, 1998; Perret *et al.*, 2001; Pacini *et al.*, 2003; Wolff *et al.*, 2003, 2006). There are similarities in nectar features between taxonomically unrelated species in connection with the pollinator type. These convergences are often seen as a result of plant adaptation to preferences, digestive abilities, or sugar intake efficiencies of specific pollinators (Stiles, 1976; Haber and Frankie, 1989; Martínez del Rio *et al.*, 1992; Baker *et al.*, 1998). Other studies show homogeneity of nectar sugar composition among phylogenetically related taxa over various pollination syndromes (Galetto *et al.*, 1998; Perret *et al.*, 2001; Galetto and Bernardello, 2003). Whether nectar features are related to the type of pollinator, or whether nectar sugar composition is a conservative feature relatively constant within taxonomically related species, or both, still remains uncertain.

Many field studies of the nectar characteristics of flowering species sharing a single pollination syndrome, carried out in natural plant communities, reveal adaptation to this specific syndrome, such as hummingbird flowers (Stiles and Freeman, 1993; Sazima *et al.*, 1996; Dzedziuch, 2001; McDade and Weeks, 2004a, b), moth flowers (Haber and Frankie, 1989), or bat flowers (Sazima *et al.*, 1999). Previous studies focusing on nectar sugar composition in phylogenetically related taxa comprising a large variety of pollination syndromes in, for example, Asteraceae (Baker and Baker, 1982), Scrophulariaceae (Elisens and Freeman, 1988), Fabaceae (Van Wyk, 1993), Solanaceae (Galetto *et al.*, 1998), Caryophyllaceae (Witt *et al.*, 1999) and Gesneriaceae (Perret *et al.*, 2001) were based primarily upon plant material from greenhouses or botanical gardens, such that the flower visitor impacts on nectar standing crop were unobserved. In an ecological context, however, decisions made by foragers are based upon rewards actually encountered (i.e. standing crop), and those are quite different from nectar volumes protected from flower visitors (McDade and Weeks, 2004a, b). Field observations are necessary to determine the role of nectar features in the interactions between plants and flower visitors. This study investigates taxonomically related species (Gentianales) all native to a tropical montane forest in southern Ecuador, under natural conditions. The monophyletic order Gentianales includes Apocynaceae, Gelsemiaceae, Gentianaceae, Loganiaceae and Rubiaceae

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(Backlund *et al.*, 2000). Rubiaceae range among the most predominant Andean families in floristic studies (e.g. Gentry, 1988; Madsen and Øllgaard, 1994; Jørgensen and León-Yáñez, 1999; Dorr *et al.*, 2000; Webster and Rhode, 2001). According to Grant and Struwe (2003), the Podocarpus National Park presents one of the greatest species diversity in *Macrocarpaea* (Gentianaceae). Besides the large number of species existing at the study site, Gentianales exhibit flowers visited by bees, flies, butterflies, hummingbirds and bats, so this order is ideal for testing nectar features. Nectar composition, volume of covered and uncovered flowers, and flower visitors of 47 taxonomically related plant species from such a hitherto data-scarce region are presented here.

MATERIALS AND METHODS

Study site and plant material

The study site 'Estación Científica San Francisco' (03°58'S, 79°04'W; 1800–3150 m a.s.l.) is located within the Eastern Cordillera of the southern Ecuadorian Andes, bordering the Podocarpus National Park, which is known as an outstanding biodiversity hotspot (Barthlott *et al.*, 1996). Most parts are covered with undisturbed or slightly disturbed montane rain forest. Detailed information on the floristic composition of the study site is provided in Bussmann (2001), Paulsch (2002) and Homeier (2004). Mean annual temperatures range from 15.5 °C in the lower areas to 9 °C at higher elevations. Annual rainfall increases from about 2000 mm in lower areas to >5000 mm in higher areas (P. Emck, University of Erlangen, Germany, unpubl. res.). Fieldwork was carried out from March to July 2000, September 2000 to February 2001 and from August to December 2001. All members of the order found at the study site were investigated except nine species of the subfamily Asclepiadoideae (Apocynaceae), which are treated in a separate paper, because their highly derived floral structure, their pollinia-forming habit demands and special pollination mechanisms. Gelsemiaceae and Loganiaceae did not occur at the study site. For Rubiaceae, the taxonomic classification of Andersson (1993) was followed and Gentianaceae were classified following Struwe *et al.* (2002). Voucher specimens are housed at MO and UBT.

Characterization of flower syndromes and observation of flower visitors

Considering the floral morphology of Gentianales, there is great variability in floral displays (corolla size, colour, scent) and nectar accessibility (corolla shape, corolla opening, tube length). The notion of pollination syndrome (Vogel, 1969; Faegri and van der Pijl, 1980; Proctor *et al.*, 1996) was used to group the species. Classification was based on a set of morphological characteristics such as corolla shape, corolla colour, scent, pattern of floral anthesis and nectar secretion. Additionally, flower visitors were observed in the field. Each plant species considered to belong to the melittophilous, myiophilous or ornithophilous syndrome was observed for at least 12 h from 0600 h to

1800 h in blocks of 4 h. Night-flowering species were observed during the day and from 1800 h until midnight.

Species were classified as myiophilous when they were visited exclusively by diptera. The criteria for melittophily were: flowers open during the day, corolla white, cream, yellow or light blue, in some cases sweet diurnal scent emission (*Faramea coerulescens*, *F. glandulosa*, *Arcytophyllum macbridei*), small corolla tubes (<15 mm) and no visitation by hummingbirds. The criteria for ornithophily were: corolla or inflorescence branch red, yellow, blue or violet, no scent and frequent visitation by hummingbirds. 'Sphingophily' is used as a generic term for all species morphologically adapted to pollination by lepidopterans (including psychophily; *Arachnothryx lojensis*). The criteria for sphingophily in the narrow sense were: synchronized anthesis at night, corolla coloured white to cream, very narrow corolla tube, sweet fragrance and scent emission beginning or becoming more intense in the evening. Finally, chiropterophily was assigned by bell-shaped corolla, mushroom-like scent being more intensive during the night, and visitation by bats.

Nectar sampling and analysis

In order to measure the nectar volumes that legitimate flower visitors may obtain, the nectar standing crop was sampled at 0600 h, 1000 h, 1400 h and 1800 h for diurnal and nocturnal uncovered flowers, and at 1800 h, 2200 h, 0200 h, and 0600 h for nocturnal uncovered flowers. To determine the daily nectar production and nectar sugar concentrations, flowers were covered at the bud stage. The nectar of bee, fly and hummingbird flowers was sampled in the evening, and that of moth and bat flowers was sampled in the early morning by inserting microcapillaries and then recording the nectar volume. An aliquot of 2 µL nectar (or less if flowers contained <2 µL nectar) was injected into Eppendorf® caps with 70 % ethanol for each flower. Nectar taken at the same time and from the same species was pooled. The samples were frozen until determination of nectar concentration and composition. For analysis, samples were dried in a vacuum centrifuge, diluted with 200 µL water, and filtered using a WATERS™ high performance carbohydrate column to avoid contamination. The injection volume was 10 µL, and elution took place with an acetonitrile–water mixture (71 : 29) at a flow rate of 1.4 mL min⁻¹ and a temperature of 35 °C. Glucose, fructose, and sucrose were detected with a refraction index detector and quantified with the WATERS Millennium Software™. Concentrations were converted from µg µL⁻¹ to sucrose-equivalent, percentage weight per total weight, using table 63 in the 50th edition of the *Handbook of Chemistry and Physics* (Weast, 1969).

Statistical analysis

Data were tested for normality and homogeneity of variance. In order to meet these criteria, nectar volume of covered flowers was log ($x + 1$) transformed, and the sugar ratio was square root transformed. When data met the assumption for parametric statistics, ANOVA followed by Tukey–Kramer HSD for unequal N were used to test

for differences of species means among classes of pollination. Because data on nectar standing crop violated the normality assumption for parametric statistics, the Kruskal–Wallis rank sums test followed by the Tukey–Kramer multiple comparison for non-parametric data were used to ascertain differences of species means among classes of pollination (Siegel and Castellan, 1988). The chord-normalized expected species shared (CNESS) distance index (Trueblood *et al.*, 1994), ranging between 0 and the square root of 2, was used to determine differences between the sampled species' nectar sugar composition. CNESS is a metric version of Grassle and Smith's NESS similarity index (Grassle and Smith, 1976), and both can be regarded as more generalized forms of the Morisita index (Morisita, 1959). These are the most appropriate indices for analysing quantitative data (Wolda, 1981, 1983; Trueblood *et al.*, 1994). Calculation of the CNESS index was performed using the updated version of the combinatorial polythetic agglomerative hierarchical clustering (COMPAH 96) program (Boesch, 1977) provided by Gallagher at UMASS/Boston (<http://www.es.umb.edu/edgwebp.htm>). Non-linear multidimensional scaling (NMDS) was used to visualize similarities among the species. Stress is a measurement that reflects the degree of deviation of NMDS distances from true matrix distances. According to Clarke (1993), stress values below 0.05 give an excellent representation with no prospect of misinterpretation. The Sørensen index, based on presence–absence data, was calculated for the floral visitors of each plant species. Euclidean distances were calculated for the nectar volumes of covered and uncovered flowers, as well as for nectar sugar concentrations. Matrix correlation tests were used to associate distance matrices (Mantel, 1967). For example, (1–Sørensen) the matrix of floral visitors can be directly compared with the dissimilarity (CNESS) matrix of nectar sugar composition, or nectar concentration (Euclidean) distances, or any other derived matrices (e.g. from nectar volume data). For the performance of matrix correlation tests, distance matrices were calculated for 46 plant species; *Palicourea* sp. was excluded because no floral visit was observed. Matrix correlation tests were performed by the program Primer™ Version 5 (Clarke and Gorley, 2001). To test correlation, Pearson correlation was used for parametric data (sugar ratio versus dimension 1 of the NMDS), and Spearman rank order correlation *R* was used for non-parametric data (nectar volumes of uncovered versus covered flowers; mean versus standard deviation of nectar volumes in covered and uncovered flowers). The data analysis software, STATISTICA™, Version 7.0 from StatSoft, Inc. (2004) was used.

RESULTS

Nectar sugar composition and concentration

Floral nectars were sucrose-dominant in all flowers classified as ornithophilous, as well as in the majority of flowers classified as sphingophilous, with sugar ratios ranging from 1.3 to 15.5 (the only exception was *Isertia laevis* with 0.7; see Table 1). Sucrose : hexose ratios below 1 were found in

bat flowers all belonging to the tribe Helieae. The nectar sugar ratio ranged from 0.1 to 13.6 within the melittophilous syndrome. Sugar composition varied markedly among myiophilous species (Table 1) from hexose-dominant (*Gentianella* sp.) to hexose-rich (*Halenia* sp.) to sucrose-rich (*Arcytophyllum filiforme*, *Psychotria aubletiana*, *Dioicodendron dioicum*) and sucrose-dominant (*Psychotria* sp.). It is worth noting that the hexose-dominant and hexose-rich species occur at elevations above 3000 m (the only exception is *Macrocarpaea harlingii*).

There is a significant sugar ratio difference between sphingophilous and myiophilous species and between sphingophilous and chiropterophilous species (ANOVA with a following *post-hoc* test; see Table 2).

Differing nectar compositions among species, based on the CNESS index are visualized using non-linear multidimensional scaling (stress = 0.014; Fig. 1). The dominant cluster was characterized by species belonging to the melitto-, ornitho- and sphingophilous syndrome; only sphingophilous *Isertia laevis*, ornithophilous *Palicourea canarina* and melittophilous members of the genus *Arcytophyllum* are separated from this cluster. Species receiving visits exclusively from dipters are well separated from the main cluster (only myiophilous *Psychotria* sp. is located within the main cluster). Chiropterophilous species belonging to the tribe Helieae are further separated from the main cluster. There is a significant negative correlation ($r = 0.395$, $t = -2.8$, $P = 0.007$; Pearson) between dimension 1 and the sugar ratio.

Sucrose concentration averaged $149 \mu\text{g} \mu\text{L}^{-1}$ in species with the melittophilous syndrome, compared with $50 \mu\text{g} \mu\text{L}^{-1}$ in those with the chiropterophilous syndrome, whereas hexose concentration was similar $81 \mu\text{g} \mu\text{L}^{-1}$ in bee flowers and $98 \mu\text{g} \mu\text{L}^{-1}$ in bat flowers (Fig. 2). Sugar proportions between ornithophilous and sphingophilous flowers are more or less equal, amounting to $98 \mu\text{g} \mu\text{L}^{-1}$ sucrose in the former and $114 \mu\text{g} \mu\text{L}^{-1}$ sucrose in the latter, while hexose concentration was $44 \mu\text{g} \mu\text{L}^{-1}$ in hummingbird flowers and $33 \mu\text{g} \mu\text{L}^{-1}$ in moth flowers (Fig. 2). Within these two types of flowers, the hexose proportion was clearly lower than in bee, bat and fly flowers. The highest hexose concentration of $146 \mu\text{g} \mu\text{L}^{-1}$ was found in flowers of the myiophilous syndrome, whose sugar proportions were the inverse to those of the melittophilous syndrome. Considering hexose only, the proportion of fructose to glucose was more or less balanced across all species. Total sugar concentrations were variable among species (Table 1), and no significant differences could be detected among syndromes (ANOVA with following Tukey–Kramer *post-hoc* test, Table 2).

Nectar sugar concentration was not significantly correlated with floral visitors ($R = 0.097$, $P = 0.077$, matrix correlation). There was, however, a slight significant correlation between nectar sugar composition and floral visitors ($R = 0.197$, $P = 0.043$, matrix correlation).

Nectar volume and standing crop

In covered flowers, nectar volumes varied markedly among the pollination syndromes and among species

TABLE 1. Tribe, flower visitors of each species investigated, sample size N_f flowers, N_i individuals, N_s pooled nectar samples analysed, nectar sugar concentration and composition, daily nectar production of covered flowers and nectar standing crop grouped according to their pollination syndrome

Species	Tribe*	Flower visitors†	N_f (N_i)	N_s	Conc. [%w/w] $\bar{x} \pm$ s.d.	% Fructose $\bar{x} \pm$ s.d.	% Glucose $\bar{x} \pm$ s.d.	% Sucrose $\bar{x} \pm$ s.d.	S/(F + G) $\bar{x} \pm$ s.d.	Nectar production 24 h covered $\bar{x} \pm$ s.d. (μ L) (n)	Standing crop 1800–0600 h $\bar{x} \pm$ s.d. (μ L) (n)	Standing crop 0600–1800 h $\bar{x} \pm$ s.d. (μ L) (n)
Myiophily												
<i>Arcytophyllum filiforme</i> (Ruiz & Pav.) Standl.	R2	Dip	3 (1)	1	32.0	30.8	36.3	33.0	0.5	0.3 ± 0.2 (3)	–	0.1 ± 0.03 (7)
<i>Diaicodendron dioicum</i> (K. Schum. & K. Krause) Taylor	R8	Dip	13 (2)	1	31.0	20.0	34.1	46.0	0.9	No data	–	0.3 ± 0.1 (13)
<i>Gentianella</i> sp. 1	G1	Dip	15 (15)	1	59.0	45.8	54.2	0.0	0.0	0.7 ± 0.5 (6)	–	0.1 ± 0.1 (15)
<i>Halenia</i> sp. 1	G1	Dip	12 (10)	2	26.8 ± 14.5	44.1 ± 9.7	42.8 ± 8.9	13.1 ± 18.5	0.2	1.1 ± 0.4 (10)	–	0.1 ± 0.1 (12)
<i>Psychotria aublletiana</i> Steyererm.	R1	Dip	3 (1)	1	13.0	25.5	28.6	45.9	0.8	No data	–	0.5 ± 0.1 (3)
<i>Psychotria</i> sp. 1	R1	Dip	16 (6)	2	25.0 ± 8.5	16.2 ± 13.8	10.5 ± 8.1	73.3 ± 21.9	4.7 ± 4.6	0.7 ± 0.4 (9)	–	0.2 ± 0.2 (14)
Melitophily												
<i>Faramaea uniflora</i> Dwyer & M. V. Hayden	R3	Hym, Dip	9 (6)	2	2.3 ± 2.5	0	0	100	Not defined	0.2 ± 0.2 (7)	–	0.2 ± 0.2 (11)
<i>Psychotria</i> <i>acuminata</i> Benth.	R1	Hym, Dip	34 (6)	1	9.0	16.2	17.3	66.5	2.0	1.9 ± 0.8 (18)	–	0.8 ± 0.4 (19)
<i>Psychotria tinctoria</i> Ruiz & Pav.	R1	Hym	6 (4)	2	49.5 ± 3.5	18.3 ± 2.5	21.3 ± 3.0	60.4 ± 5.4	1.5 ± 0.3	4.9 ± 3.4 (6)	–	0.8 ± 0.7 (6)
<i>Palicourea</i> sp. nov. ined. C.M. Taylor	R1	Hym	78 (8)	3	24 ± 5.6	16.6 ± 1.4	16.5 ± 1.3	66.9 ± 2.5	2.0 ± 0.2	5.8 ± 2.3 (78)	–	0.1 ± 0.3 (25)
<i>Coccyzselum condalia</i> Pers.	R7	Hym	27 (8)	2	13.7 ± 11.7	5.7 ± 5.2	6.8 ± 6.0	87.5 ± 11.2	4.5 ± 1.2	0.8 ± 0.4 (15)	–	0.3 ± 0.2 (27)
<i>Manettia</i> sp. 2	R2	Hym	23 (3)	1	22.0	9.0	8.4	82.6	4.7	3.1 ± 1.4 (23)	–	0.8 ± 1.2 (23)
<i>Rudgea ciliata</i> (Ruiz & Pav.) Spreng	R1	Hym	25 (4)	2	40.8 ± 14.5	12.7 ± 4.8	13.3 ± 7.6	73.9 ± 12.4	3.3 ± 2.0	2.1 ± 0.9 (11)	–	0.6 ± 0.5 (22)
<i>Stilpnophyllum oelgaardii</i> L. Andersson	R4	Hym	45 (7)	6	27.4 ± 4.5	4.9 ± 1.4	15.8 ± 1.2	79.2 ± 2.5	3.9 ± 0.6	3.9 ± 2.2 (62)	–	1.2 ± 0.9 (20)
<i>Arcytophyllum machbridei</i> Standl.	R2	Hym	27 (6)	3	20.8 ± 11.6	30.9 ± 14.7	30.9 ± 19.7	38.1 ± 34.2	0.6 ± 1.0	0.5 ± 0.2 (12)	–	0.3 ± 0.3 (35)
<i>Arcytophyllum capitatum</i> (Benth.) K. Schum.	R2	Hym, Col	40 (14)	5	38.3 ± 12.9	38.8 ± 3.3	44.0 ± 7.0	17.2 ± 10.2	0.2 ± 0.1	1.0 ± 0.5 (24)	–	0.2 ± 0.1 (40)
<i>Arcytophyllum ciliolatum</i> Standl.	R2	Hym, Col	45 (12)	6	41.5 ± 20.8	31.8 ± 5.9	32.0 ± 5.7	36.1 ± 11.5	0.6 ± 0.3	1.4 ± 0.8 (13)	–	0.6 ± 0.7 (31)
<i>Arcytophyllum thymifolium</i> (Ruiz & Pav.) Standl.	R2	Hym, Col	8 (4)	2	22 ± 21.2	44.8 ± 0.4	42.8 ± 1.5	12.4 ± 1.9	0.1 ± 0.02	0.4 ± 0.2 (7)	–	0.3 ± 0.2 (8)
<i>Arcytophyllum vernicosum</i> Standl. sp. nov. ined. C.M. Taylor	R2	Hym, Col, Dip	12 (3)	2	22 ± 2.8	22.5 ± 2.1	24.0 ± 0.2	53.5 ± 2.2	1.2 ± 0.1	0.4 ± 0.4 (13)	–	0.4 ± 0.2 (12)
<i>Notopleura vargasiana</i> Poepp. & Endl.	R1	Hym, Lep, Dip	21 (7)	3	26.5 ± 25.3	6.4 ± 4.3	5.6 ± 3.8	88.0 ± 8.0	13.6 ± 14.5	0.8 ± 0.3 (10)	–	0.3 ± 0.3 (21)
<i>Faramaea cf. glandulosa</i> K. Schum. & K. Krause	R3	Hym, Lep	8 (4)	2	41.5 ± 9.2	14.3 ± 9.8	13.6 ± 9.7	72.1 ± 19.5	3.8 ± 3.3	1.6 ± 0.8 (12)	–	0.4 ± 0.2 (12)
<i>Psychotria reticulata</i> Ruiz & Pav.	R1	Hym, Lep	19 (5)	3	25.3 ± 14.0	12.7 ± 0.5	16.4 ± 2.6	70.9 ± 2.1	2.4 ± 0.3	1.0 ± 0.5 (27)	–	0.3 ± 0.2 (12)
<i>Faramaea coerulescens</i> K. Schum. & K. Krause	R3	Hym, Lep, T3	8 (3)	1	14.5	23.2	17.1	59.8	1.5	4.7 ± 2.6 (8)	–	0.9 ± 0.7 (10)
Ornithophily												
<i>Palicourea angustifolia</i> Kunth	R1	T, Hym, Lep	24 (6)	8	19.8 ± 5.9	14.4 ± 2.2	12.4 ± 2.7	73.2 ± 4.7	2.8 ± 0.7	7.7 ± 2.6 (24)	–	2.2 ± 1.7 (53)
<i>Palicourea calycina</i> Benth.	R1	T, Hym, Lep	44 (12)	9	24.9 ± 10.5	18.3 ± 5.9	16.0 ± 7.5	65.8 ± 13.2	2.2 ± 0.8	16.1 ± 5.1 (44)	–	1.1 ± 1.0 (49)
<i>Palicourea carmarina</i> C.M. Taylor	R1	T, Hym, Lep	106 (14)	3	14.0 ± 2.0	29.3 ± 3.7	15.1 ± 3.8	55.6 ± 6.3	1.3 ± 0.3	14.4 ± 9.6 (106)	–	5.6 ± 7.0 (62)
<i>Palicourea heterochroma</i> K. Schum. & K. Krause	R1	T, Hym, Lep	21 (6)	13	15.3 ± 4.9	18.2 ± 5.4	14.4 ± 5.3	67.4 ± 10.6	2.3 ± 0.9	43.1 ± 16.3 (21)	–	4.0 ± 4.8 (29)
<i>Palicourea luteonivea</i> C.M. Taylor	R1	T, Hym, Lep	26 (8)	16	15.9 ± 4.0	14.8 ± 4.0	10.8 ± 8.6	74.4 ± 12.5	3.3 ± 0.8	15.9 ± 7.1 (26)	–	2.8 ± 3.4 (92)
<i>Palicourea submontosa</i> (Ruiz & Pav.) DC	R1	T2, Hym, Lep	15 (4)	2	17.0 ± 5.7	14.2 ± 3.8	12.2 ± 4.8	73.6 ± 8.6	3.0 ± 1.3	1.6 ± 0.6 (15)	–	0.6 ± 0.5 (15)

<i>Palticourea</i> c.f. <i>weberbaueri</i> K. Krause	R1	T, Hym, Lep	33 (10)	23	21.8 ± 8.4	20.4 ± 7.5	20.8 ± 11.2	58.9 ± 18.0	1.7 ± 0.8	7.5 ± 3.8 (45)	–	1.6 ± 2.9 (92)
<i>Palticourea lobbii</i> Standl.	R1	T, Hym	13 (5)	3	17.7 ± 2.1	11.8 ± 0.8	7.7 ± 0.7	80.5 ± 0.8	4.1 ± 0.2	10.6 ± 3.7 (15)	–	3.6 ± 4.0 (15)
<i>Palticourea lyristsipula</i> Wernham	R1	T, Hym	49 (10)	14	26.1 ± 11.7	18.0 ± 6.6	17.6 ± 8.3	64.3 ± 14.7	2.1 ± 1.0	4.8 ± 2.0 (57)	–	0.8 ± 1.3 (154)
<i>Palticourea thysiflora</i> SW	R1	T, Hym	35 (6)	13	10.6 ± 2.6	22.8 ± 6.7	17.7 ± 5.8	59.5 ± 12.3	2.1 ± 2.6	30.6 ± 15.7 (35)	–	2.8 ± 2.9 (51)
<i>Manettia</i> sp. 1	R2	T	12 (4)	4	8.3 ± 2.4	15.9 ± 6.5	7.3 ± 4.9	76.8 ± 11.3	4.1 ± 2.5	51.9 ± 7.1 (12)	–	1.7 ± 2.4 (38)
<i>Symbolanthus calygonus</i> (Ruiz & Pav.) Griseb. ex Gilg	G2	T	10 (5)	1	15.5	20.1	8	71.9	2.6	48.9 ± 16.5 (10)	–	6.8 ± 5.7 (15)
<i>Palticourea</i> sp. 1	R1	–	11 (1)	1	14	17.8	17.6	64.6	1.8	14.5 ± 4.5 (11)	–	0.5 ± 0.7 (11)
Psycho-Sphingo-Phalaenophil												
<i>Arachnothryx tojensis</i> Steyerl.	R6	Lep	17 (5)	11	15.5 ± 4.8	7.6 ± 3.6	3.0 ± 5.3	89.4 ± 8.3	10.3 ± 4.6	1.8 ± 0.9 (17)	–	0.6 ± 1.1 (248)
<i>Ladenbergia</i> sp. 1	R4	Noc, Sph, T	3 (2)	1	22	12.2	6.4	81.4	4.4	45.2 ± 9.7 (3)	–	1.1 ± 1.0 (5)
<i>Palticourea andrei</i> Standl.	R1	Noc, Sph, T	5 (2)	1	17	16	20.2	63.7	1.8	14.7 ± 3.1 (5)	–	1.5 ± 1.0 (4)
<i>Iseritia laevis</i> (Triana) B. M. Boom	R9	Noc, Sph, T, Hym	28 (6)	18	17.9 ± 6.8	33.6 ± 9.0	31.3 ± 7.1	35.1 ± 12.2	0.7 ± 0.9	39.3 ± 19.6 (28)	–	4.7 ± 7.2 (119)
<i>Ladenbergia</i> sp. 2	R4	Noc, Sph, Hym	10 (1)	1	9.5	14.5	17.6	67.9	2.1	5.4 ± 1.7 (10)	–	0.2 ± 0.3 (15)
<i>Hillia parasitica</i> Jacquin	R5	Sph, Hym	4 (2)	1	16	2.5	3.6	93.9	15.5	41.0 ± 9.9 (3)	–	24.3 (1)
<i>Hillia wurdackii</i> Steyerl.	R5	Sph,	3 (2)	1	19.5	6.5	2.5	91	10.1	38.3 ± 15.2 (4)	–	29.5 (1)
Chiropterophily												
<i>Macrocarpaea arborescens</i> Gilg	G2	G1, T, Hym	7 (4)	1	23	43.5	29.6	26.9	0.4	67.9 ± 15.8 (7)	–	1.6 ± 1.7 (14)
<i>Macrocarpaea hartlingii</i> J. S. Pringle	G2	G1, T, Hym, Noc	13 (6)	1	11	36.3	35.3	28.4	0.4	73.1 ± 27.8 (13)	–	2.5 ± 2.5 (19)
<i>Macrocarpaea noctiluca</i> J. R. Grant & Struwe	G2	G1, T, Hym, Spi	12 (6)	1	10.5	33.9	25.8	40.3	0.7	98.8 ± 28.3 (12)	–	4.4 ± 12.6 (26)
<i>Symbolanthus</i> sp. nov. ined.	G2	Noc, Lep, Dip G1, T, Hym, Spi, Noc	12 (5)	1	12	34.9	19.2	45.9	0.8	102.9 ± 42.1 (15)	–	9.5 ± 6.2 (15)

Flower visitors were observed by the author with the exception of those observed by ¹ Matt (2001), ² Dziedziuch (2001) and ³ A. Paulsch (pers. comm.).

\bar{x} = mean; s.d. = standard deviation; *n* = number of flowers sampled for nectar volumes.

* G, Gentianeaceae; R, Rubiaceae. Tribal affiliation is designated by the following numerals: G1, Gentianeae; G2, Helieae; R1, Psychotriaceae; R2, Hedyotideae; R3, Coussareae; R4, Cinchoneae; R5, Hillieae; R6, Rondeletieae; R7, Coccocypseleae; R8, Condamineae; R9, Iseritiae.

G, Glossophagidae; T, Trochilidae; Hym, Hymenoptera (mainly Apidae); Lep, Lepidoptera except Sphingidae, Noctuidae, Geometridae; Sph, Sphingidae; Noc, Noctuidae and Geometridae; Dip, Diptera; Col, Coleoptera.

TABLE 2. Means and standard deviation of nectar sugar concentration and sugar ratio, nectar volume covered and nectar standing crop of flowers in different pollination syndromes

Syndrome	<i>n</i>	Conc. (%w /w) $\bar{x} \pm$ s.d.	Sugar ratio S/(F + G) $\bar{x} \pm$ s.d.	Nectar volume 24 h covered (μ L) $\bar{x} \pm$ s.d.	Standing crop 0600–1800 h (μ L) $\bar{x} \pm$ s.d.	Mann–Whitney <i>U</i> Nectar volume covered vs. standing crop
Miophilous	6 ¹	31.1 \pm 15.2	1.2 \pm 1.8 ^a	0.7 \pm 0.3 ^{a,b,c}	0.2 \pm 0.2 ^{a,b,c}	$z = 2.2$, $P = 0.025$
Melittophilous	17 ²	25.9 \pm 12.8	2.9 \pm 3.2	2.0 \pm 1.8 ^{e,g,h}	0.5 \pm 0.3 ^{d,e}	$z = 2.3$, $P = 0.021$
Ornithophilous	13	17.0 \pm 5.1	2.6 \pm 0.9	20.6 \pm 17.2 ^{a,d,e}	2.6 \pm 1.9 ^{a,d}	$z = 3.4$, $P = 0.000$
Sphingophilous (including one psychophilous species)	7	16.8 \pm 3.9	6.4 \pm 5.6 ^{a,b}	26.5 \pm 18.5 ^{b,f,g}	8.8 \pm 12.5 ^b	$z = 3.8$, $P = 0.000$
Chiropterophilous	4	14.1 \pm 5.9	0.6 \pm 0.2 ^b	85.7 \pm 17.7 ^{c,d,f,h}	4.5 \pm 3.5 ^{c,e}	$z = -2.2$, $P = 0.025$
ANOVA/K–W ANOVA		$F_{4,42} = 3.7$ $P = 0.011$	$F_{4,41} = 4.3$ $P = 0.005$	$F_{4,40} = 29.3$ $P = 0.000$	$H(4, 7) = 27.4$ $P = 0.000$	

Mann–Whitney *U*-test of significant differences between covered nectar volumes and standing crop.

n = number of species, ¹number of species nectar production: *n* = 4 miophilous syndrome; ²number of species sugar ratio: *n* = 16 melittophilous syndrome.

A same letter following the values indicates significantly different pairs after ANOVA with following Tukey–Kramer HDS *post-hoc* test ($\alpha = 0.05$) or Kruskal–Wallis ANOVA with Tukey–Kramer *post-hoc* test ($\alpha = 0.05$).

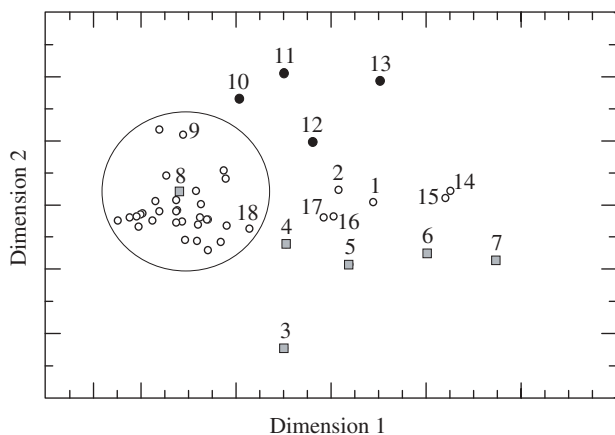


FIG. 1. Non-linear multidimensional scaling (NMDS) of the nectar sugar composition of 47 species based on the CNESS index. 1, *Isertia laevis*; 2, *Palicourea canarina*; 3, *Dioicodendron dioicum*; 4, *Psychotria aubleitiana*; 5, *Arcytophyllum filiforme*; 6, *Halenia*; 7, *Gentianella*; 8, *Psychotria* sp.; 9, *Symbolanthus calygonous*; 10, *Macrocarpaea harlingii*; 11, *Sym.* sp.; 12, *M. noctiluca*; 13, *M. arborescens*; 14, *A. thymifolium*; 15, *A. capitatum*; 16, *A. ciliolatum*; 17, *A. macbridei*; 18, *A. vernicosum*. Squares, miophilous species; filled circles, chiropterophilous species.

with the same syndrome, ranging from 0.3 to 1.1 μ L in fly, 0.2 to 5.8 μ L in bee, 1.6 to 51.9 μ L in hummingbird, 67.9 to 102.9 μ L in bat and 1.8 to 45.2 μ L in moth flowers (Table 1). Daily nectar production differed significantly among the pollination syndromes, except for ornithophilous versus sphingophilous and miophilous versus melittophilous (ANOVA, with following Tukey–Kramer *post-hoc* test; see Table 2). In uncovered flowers sampled during the day, nectar volumes among the pollination syndromes and among species with the same syndrome were less variable, and ranged from 0.1 to 0.5 μ L in fly, 0.1 to 1.2 μ L in bee, 0.5 to 6.8 μ L in hummingbird, 1.6 to 9.5 μ L in bat and 0.2 to 29.5 μ L in moth flowers (Table 1). The nectar standing crop measured during the day differed significantly between miophilous flowers versus ornithophilous, sphingophilous, and chiropterophilous flowers, and between melittophilous flowers versus ornithophilous and chiropterophilous flowers (Table 2).

A significant correlation was found between the nectar volumes of covered flowers and floral visitors ($R = 0.228$, $P = 0.007$, matrix correlation test). Standing crop and floral visitors were also significantly correlated ($R = 0.157$, $P = 0.028$, matrix correlation test).

The nectar standing crop sampled during the day was significantly correlated with nectar volumes of covered flowers (Spearman coefficient $R = 0.83$, $P = 0.000$). Conversely, diurnal standing crop values differed significantly from covered nectar volumes in all syndromes (Mann–Whitney *U*-test; Table 2). The nectar standing crop of bat and moth flowers sampled at night did not differ significantly (Mann–Whitney *U*-test: bat flowers $z = -1.7$, $P = 0.08$, moth flowers $z = 0.8$, $P = 0.42$) from those of covered flowers, which indicates a low nocturnal visitation rate.

Among species, the distribution of variability of nectar volumes measured by the coefficient of variation (standard deviation/mean) is shown in Fig. 3. Nectar volumes of uncovered flowers sampled during the day were more variable than the nectar volumes sampled at night and the nectar volumes of covered flowers. Coefficients of variation among nectar volumes in uncovered nocturnal flowers ranged within those for covered flowers, further indicating a low nocturnal visitation rate. There was a significant linear correlation between the means and standard deviations of nectar volumes of covered and uncovered flowers sampled during the day (Spearman coefficient $R = 0.98$, $P = 0.000$; $R = 0.94$, $P = 0.000$, respectively).

DISCUSSION

Nectar sugar composition and concentration

The main goal of this study was to determine whether nectar features are related to the type of pollinator. Regarding the pollination syndromes, no nectar sugar concentration correlation was found. Regarding sugar composition, the only significant differences were found in chiropterophilous and miophilous flowers, which had a significantly lower sugar ratio than sphingophilous flowers. This is further substantiated by the NMDS of the CNESS dissimilarity index

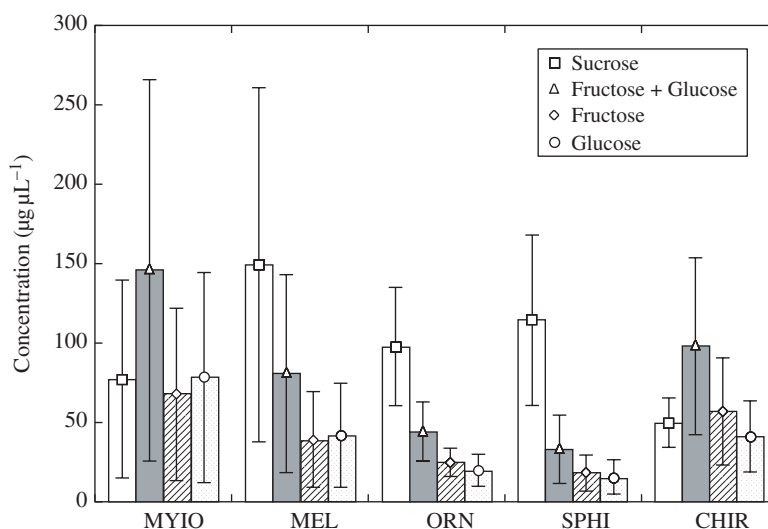


FIG. 2. Mean proportion of sucrose and hexose (fructose + glucose) in the nectar of 47 Gentianales species arranged according to their pollination syndromes: MYIO, myiophilous syndrome ($n = 6$); MEL, melittophilous syndrome ($n = 17$); ORN, ornithophilous syndrome ($n = 13$); SPHI, sphingophilous syndrome ($n = 7$); CHIR, chiropterophilous syndrome ($n = 4$). Vertical bars represent s.d.

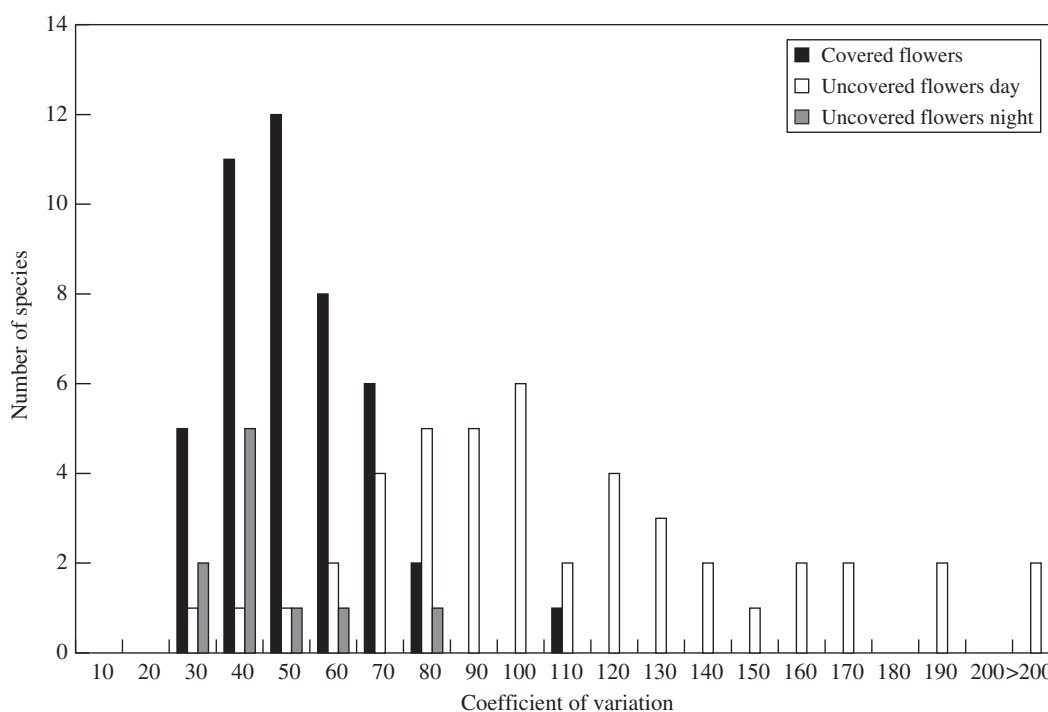


FIG. 3. Distribution of variability, measured by the coefficient of variation of nectar volume of covered flowers (45 plant species) and uncovered flowers (ten species probed during the night, and 45 species probed during the day).

based on nectar sugar compositions (Fig. 1). Nectars from flowers visited by hummingbirds, bees, butterflies and moths formed one homogenous cluster, and nectar from flowers visited exclusively by flies (except *Psychotria* sp.) formed a second group. A third group included nectar from flowers visited by bats. Although sucrose is the predominant floral nectar sugar in 41 out of 47 investigated species, nectars from species within each pollination

syndrome tend to have characteristic sugar compositions (Fig. 2). In this study, sucrose was the dominant constituent in all flowers of the ornithophilous syndrome. Ornithophilous flowers of several taxonomic groups have sucrose-dominant nectar (Baker and Baker, 1983; Freeman *et al.*, 1984; Gottsberger *et al.*, 1984; Stiles and Freeman, 1993; Dzedziuch, 2001; Perret *et al.*, 2001; Galetto and Bernardello, 2003). Nectar of the ornithophilous plants

investigated contained, on average, 68.2% sucrose, agreeing with the results of Nicolson and Fleming (2003), who showed that hummingbird nectars cluster around 64% sucrose. Considering the sugar concentration in the nectar of Gentianales, values for hummingbird flowers (17.0%) were slightly lower than those found in the literature, citing a range from 21 to 26% (Baker, 1975; Waser and Pyke, 1981; Heyneman, 1983; Stiles and Freeman, 1993; Sazima *et al.*, 1996; Kraemer, 1998; Perret *et al.*, 2001; McDade and Weeks, 2004a). However, feeding experiments with hummingbirds show, that even more concentrated sugar solutions (31–45%) are preferred (Pyke and Waser, 1981; Kingslover and Daniel, 1983; Tamm and Gass, 1986; Roberts, 1996; Nicolson and Fleming, 2003).

This study's data on the sugar composition of sphingophilous Rubiaceae agree with the studies of several other families (Baker and Baker, 1983; Haber and Frankie, 1989; Schwerdtfeger, 1996), showing sucrose-dominant nectar presence in the majority of sphingophilous flowers. Sphingophilous flowers produced less concentrated nectar (16.8%) than the reported mean of 21% (Haber and Frankie, 1989), and 19% (hawkmoths) and 22% (settling moths) reviewed by Heyneman (1983).

The nectar of the majority (13 of 17 species) of melittophilous flowers is sucrose-dominant. This agrees with previous observations for melittophilous flowers of the Antirrhineae (Scrophulariaceae; Elisens and Freeman, 1988), Iridaceae (Goldblatt *et al.*, 1998) and Sinningieae (Gesneriaceae; Perret *et al.*, 2001). Hexose-dominant to sucrose-rich nectar is found in four species of *Arcytophyllum* (Rubiaceae). According to the phylogeny of the genus *Arcytophyllum* provided by Andersson *et al.* (2002) and Wolff and Liede-Schumann (2006), the most derived species of *A. macbridei* and *A. vernicosum* have higher sucrose proportions, whereas the basal *A. thymifolium* has a very low sucrose/hexose ratio. This suggests a tendency towards a higher percentage of sucrose in the genus. Nectar concentration of the flowers of the melittophilous syndrome studied here are lower (25.9%) than the corresponding values in the temperate and tropical regions reported by Pyke and Waser (1981) (36%) and Galetto *et al.* (1998) (48%), but are close to the value (29%) for melittophilous Gesneriaceae (Perret *et al.*, 2001). Bees prefer very concentrated nectar to guarantee energetically profitable foraging (Bolten and Feinsinger, 1978).

Distinctive nectar composition is associated with the chiropterophilous syndrome, in which particularly low sucrose production is responsible for hexose dominance (Fig. 2). The high hexose proportion found in flowers of *Macrocarpaea* corresponds well with other bat flowers (Baker and Baker, 1983; Baker *et al.*, 1998; Perret *et al.*, 2001). Nectar concentration (14%) of this study's bat-pollinated Gentianaceae corresponds with the chiropterophilous Gentianaceae (10–15%) reported by Machado *et al.* (1998), as well as with the results from Sazima *et al.* (1999) who reported an average sugar concentration of bat-pollinated flower assemblages of 15% (lowland) and 18% (highland). These values are close to the median range of the frequency distribution reviewed by Helversen (1993) for 33 species of neotropical bat-pollinated flowers. Roces *et al.*

(1993), however, showed in a series of dual choice tests that glossophagine bats preferred higher nectar concentrations up to 50%.

Flies prefer hexose-dominant and hexose-rich nectars (Baker and Baker, 1983). High hexose proportions are found in flowers exclusively visited by flies. Sugar concentration (31%) varied markedly within the myiophilous syndrome (range 13–59%). Pombal and Morellato (1995) found very low sugar concentrations (2%) in fly-pollinated Araliaceae. Machado and Loiola (2000) report 16% in *Cordia* (Boraginaceae) and 30% in *Borreria* (Rubiaceae).

Except for the nectar of *Psychotria aubletiana*, nectars analysed for 21 species of tribe Psychotrieae are quite homogenous and sucrose-dominant, even though species are morphologically classified as ornithophilous, melittophilous, sphingophilous and myiophilous. In contrast, working with two other ornithophilous Ecuadorian species of the tribe, Bernardello *et al.* (1994) found hexose-rich and hexose-dominant nectar. The sugar compositions among Gentianales reported here indicate sucrose-dominant (53.5–100% sucrose) or sucrose-rich (33–46% sucrose) nectars predominate, even though flies, bees, beetles, diurnal and nocturnal butterflies, hummingbirds and bats were the principal floral visitors. Only *Arcytophyllum capitatum*, *A. thymifolium*, *Macrocarpaea harlingii*, *M. arborescens*, *Halenia* sp. and *Gentianella* sp. had hexose-rich to hexose-dominant nectar (0–28.4% sucrose). The homogeneity of nectar sugar composition in the majority of species indicates that this is a conservative characteristic in the Gentianales investigated. The data support hypotheses of phylogenetic constraint on nectar sugar composition. Interestingly, similar results are found in different families if nectar sugar composition is compared with flower morphology and studied within small monophyletic groups (Elisens and Freeman, 1988; Galetto *et al.*, 1998; Perret *et al.*, 2001; Torres and Galetto, 2002).

In general, no correlation of floral visitors to nectar concentration was found (matrix correlation). A weak significant correlation was found, however, between floral visitors and nectar sugar composition (matrix correlation). It is likely that there has been some degree of an adaptive convergence of floral nectar compositions to principal pollinator type within the constraints set by phylogenetic history.

Nectar volume and standing crop

The nectar volumes of covered flowers are related to, and differ significantly among, pollination syndromes, with the exception of ornithophilous versus sphingophilous and myiophilous versus melittophilous flowers.

In this study, bat flowers contained about half of the average nectar volume found by Sazima *et al.* (1999) (151 µL lowland, 167 µL highland). The nectar volume of seven bat-visited flowers studied by Tschapka (2004) varied from 100 to 21260 µL. However, Perret *et al.* (2001) reported an average amount of 89 µL for two chiropterophilous Sinningieae (Gesneriaceae), and Machado *et al.* (1998) reported for the Gentianaceae *Irlbachia* an average nectar amount of 43 µL. Nevertheless, the bat

flowers investigated here contained four times as much nectar as the hummingbird flowers studied. Mean nectar amounts from the ornithophilous flowers fell within the range of other neotropical bird-visited flowers, with 16.9 μL (Sazima *et al.*, 1996), 28.9 μL (Kraemer, 1998), 16.3 μL (Schmitt, 2000), 18.4 μL (Perret *et al.*, 2001), 38.5 μL (Dziedziuch, 2001) and 8.8 to 72.7 μL (McDade and Weeks, 2004a) being reported. Haber and Frankie (1989) observed highly variable nectar volumes among sphingophilous species with a mean of approx. 60 μL , which is twice the mean nectar volume found in this study. Low nectar volumes have generally been found in melittophilous flowers; however, Perret *et al.* (2001) found more than ten times more nectar (15.4 μL) in flowers of bee-pollinated Gesneriaceae than in the bee flowers studied here. Mean nectar volumes below 1 μL were found in myiophilous species. In addition, there is a significant correlation between floral visitors and covered and uncovered nectar volumes (matrix correlation).

The nectar volumes of covered flowers have little relation to the standing crop quantities actually offered to potential flower visitors (as this study shows by the significantly lower values in standing crop compared with the cumulative nectar of covered flowers, and by the results of McDade and Weeks, 2004b), but even the standing crop nectar volumes differed among the syndromes. On the other hand, there is a positive correlation between nectar sampled during the day in covered and uncovered flowers. According to Zimmermann (1988), there must be a significant relationship between nectar production and standing crop if pollinators are to exert any selective pressure on the rate of nectar production. The amount of nectar obtained by the pollinators from the standing crop is determined by nectar production, as well as by depletion and by the morphological match between the pollinator and the flower (Rathcke, 1992). Environmental factors such as temperature, relative humidity and soil moisture also affect standing crop nectar. The data reveal great variability in the coefficients of variation (CV) for nectar volume among plant species, and even greater variability in the CV for the diurnal nectar standing crop. Variability in nectar amount is quite common (Rathcke, 1992; Petanidou and Smets, 1995; Cresswell, 1998; McDade and Weeks, 2004a, b). Foragers are sensitive to the CV of the reward (review in Real and Caraco, 1986; Kacelnik and Bateson, 1996; Bateson, 2002; Shafir *et al.*, 2003). Among other things, a pollinator's behaviour is influenced by the CV of the nectar standing crop, i.e. the higher the CV, the stronger the risk-aversion (Bateson, 2002; Shafir *et al.*, 2003). The linear correlation between the mean and standard deviation of nectar volume and standing crop between plant species found in this study is in accordance with the findings of Petanidou and Smets (1995) and McDade and Weeks (2004a, b).

Nectar volume influence pollinators' behaviour, which governs pollen receipt and donation (for example, see Ladio and Aizen, 1999; Manetas and Petropoulou, 2000; Lasso and Naranjo, 2003; Wolff *et al.*, 2006). Effective pollination is guaranteed when nectar reward is abundant enough to attract the pollinator but small enough to force

the pollinator to visit various individuals. Nectar volume production is therefore important in floral evolution and probably influenced by the most effective pollinator.

In summary, sucrose is the predominant floral nectar sugar in the order Gentianales. The homogeneity of nectar sugar composition in the majority of species indicates that this is a conservative characteristic in the Gentianales investigated. There is no correlation between sugar concentration and pollination syndromes. Nectar sugar composition does not differ significantly among the pollination syndromes (two exceptions being sphingophilous versus chiropterophilous and myiophilous nectars); only nectar volumes are related to pollination syndromes. Although certain nectar compositions and concentrations may be preferred by a given visitor, the results of the study show that various compositions and concentrations are accepted and tolerated by the visitor, not unlike the feeding behaviour of other species, including our own. However, some degree of an adaptive convergence of floral nectar compositions to principal pollinator type within the constraints set by phylogenetic history is likely. The driving force to visitation appears to be the volume of nectar the visitor can expect to consume. As the data on nectar volumes disclose, nectar production is important in floral evolution and influenced by the predominant pollinator.

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