

## Nectar Concentration and Composition of 26 Species from the Temperate Forest of South America

VANINA R. CHALCOFF<sup>1,\*</sup>, MARCELO A. AIZEN<sup>1</sup> and LEONARDO GALETTO<sup>2</sup>

<sup>1</sup>Laboratorio Ecotono, Universidad Nacional del Comahue, Quintral 1250, 8400, Bariloche, Argentina and <sup>2</sup>Instituto Multidisciplinario de Biología Vegetal (CONICET–UNC), Casilla de Correo 495, 5000, Córdoba, Argentina

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- **Background and Aims** Floral nectar concentration and chemical composition of 26 plant species native to the temperate forest of southern South America are reported and the relationships with the flower type are evaluated.
- **Methods** Nectar concentration was measured with a hand refractometer and sugar composition was analysed by gas–liquid chromatography. Plant species were classified into flower type categories based not only on floral features but also on data from the literature and field observations on their pollinators.
- **Key Results** Most data on nectar are new reports at the generic and/or specific level. Plant species in which more than one population was studied showed significant among-population variation in nectar sugar concentration and composition. Results showed a weak relationship between nectar traits and flower type. Many species had nectar containing 50% or more sucrose (17 of 26 species), independent of the main pollinator.
- **Conclusions** Considering that (a) nectar characteristics did not show a clear association with different flower types or with plant taxonomic membership, and (b) different populations of the same species showed large variability in sugar composition, the results suggest that other factors (e.g. historical and environmental) could be involved in determining the sugar composition of the highly endemic plant species from this region.

**Key words:** Nectar sugar composition, sugar concentration, flower type, pollination, hummingbirds, diurnal insects, nocturnal insects, temperate forest of southern South America.

### INTRODUCTION

Biotic pollination, the most common mutualism in terrestrial communities, represents an important source of reciprocal change between plants and animals (Willson *et al.*, 1996). Over evolutionary time, this interaction has contributed to the modelling of many characteristics in both flowers and pollinators (e.g. Faegri and van der Pijl, 1979; Baker and Baker, 1983; Johnson and Steiner, 2000). From the plant side, selection forces should act to maximize their attraction to pollinators, which transfer compatible pollen and enhance ovule fertilization.

To attract pollinators, plants offer different types of rewards. However, floral nectar represents the main plant reward for many pollinators and thus a putative primary selection target (e.g. Percival, 1961; Baker and Baker, 1975, 1983; Kevan and Baker, 1999). Nectar is basically a sugar solution composed of one disaccharide (sucrose) and two hexoses (glucose and fructose). Particularly, nectar is expected to diverge among different plant lineages and thus differ among taxa because different pollinators show preferences for solutions of different viscosity and/or sugar composition (e.g. Baker and Baker, 1975; Martínez del Río *et al.*, 1992; Temeles and Kress, 2003). If this is true, convergent nectar features (i.e. concentration and/or sugar composition) present in plant taxa from different lineages may represent adaptations to the behaviour, morphology and nutrition requirements of a particular pollinator type. On the other hand, divergent nectar features can be found in plant taxa from the same lineage that have maintained a close relationship with different pollinator guilds.

Two main trends can be outlined based on worldwide data from different floras: (1) nectar sugar concentration is generally higher in insect- than vertebrate-pollinated species (e.g. Cruden *et al.*, 1983; Gottsberger *et al.*, 1984; Freeman and Worthington, 1985; Proctor *et al.*, 1996); and (2) nectar sugar composition of species pollinated by hummingbirds, moths and long-tongued bees is dominated by sucrose, whereas nectar of species pollinated by passerines, short-tongued bees and neotropical bats is dominated by hexoses (Baker and Baker, 1983, 1990; Elisens and Freeman, 1988; Martínez del Río *et al.*, 1989, 1992; van Wyk, 1993; Proctor *et al.*, 1996; Baker *et al.*, 1998; Galetto and Bernardello, 2003; Nicolson and Fleming, 2003; Dupont *et al.*, 2004). However, several authors have pointed out that nectar characteristics can be highly conservative traits and that some species differing in pollinator type may show similar nectar sugar composition due to their close phylogenetic relationship (Elisens and Freeman, 1988; van Wyk, 1993; van Wyk *et al.*, 1993; Galetto *et al.*, 1998; Hingston and Mc Quillan, 2000; Perret *et al.*, 2001; Galetto and Bernardello, 2003).

The temperate forest of southern South America extends from 35°S to 55°S latitude and from the Pacific Ocean to the eastern slopes of the Patagonian Andes. This forest is characterized by one of the highest incidences of biotic pollination, particularly bird pollination, compared with other temperate biomes worldwide (Armesto and Rozzi, 1989; Riveros, 1991; Willson *et al.*, 1996; Aizen and Ezcurra, 1998). In this biome, 85% of the genera of woody plants are visited and presumably pollinated by animals, and nearly 20% are pollinated by a single resident hummingbird, *Sephanooides sephanooides* (Smith-Ramírez, 1993;

\* For correspondence. E-mail vchalcoff@crub.uncoma.edu.ar

Armesto *et al.*, 1996; Fraga *et al.*, 1997). The high incidence of pollination mutualisms is similar to what is found in more tropical latitudes but, unlike their tropical counterparts, the flora of the temperate forest of South America interacts with a relatively low number of animal pollinators (Smith-Ramírez, 1993; Armesto *et al.*, 1996; Aizen *et al.*, 2002). In addition, the flora of this region exhibits one of the highest rates of endemisms of any continental flora with many exclusive genera and even families (Aizen and Ezcurra, 1998). Thus, this temperate flora makes it an interesting system for evaluating relationships between nectar sugar concentration, nectar sugar composition and pollinator type.

In this paper, the following are analysed: (a) the nectar sugar concentration and composition of 26 species native to the temperate forest of southern South America; and (b) the relationships between nectar characteristics and flower types (based on flower morphology and main pollinators); also the amount of inter-population variation in nectar traits is assessed for some of the species. This is the first work of this kind in the temperate forest of southern South America, and the nectar-sugar composition data compiled in this study are first reports for most species.

## MATERIALS AND METHODS

Nectar was sampled from natural populations of 26 animal-pollinated plant species (out of 18 families) native to the temperate forest of South America, during the spring and summer seasons of 1997–2000. All species begin flowering during the austral spring with the exception of *Tristerix corymbosus* that flowers from autumn to spring, and through the winter (Table 1). The 26 species included in this study were selected to represent the entire range of life forms (trees, shrubs, vines, herbs, epiphytes and hemiparasites), most of the flower types (which reflect associations with different pollinator assemblages), and the proportion of bird- and insect-pollinated genera suggested by Aizen and Ezcurra (1998) for this biome (i.e. about 30 and 70 %, respectively). Plant species, populations studied, and the number of individuals and flowers sampled per population are listed in Table 1.

Nectar was extracted with capillary glass tubes from one to five flowers per individual and placed together on Whatman No. 1 chromatography paper (i.e. nectar samples obtained from different flowers of an individual were pooled for chromatographic analysis). The number of flowers sampled per plant was variable because of differences in the number of open flowers available among individuals of the same species and among species (Table 1). Likewise, the number of plants sampled per population was variable because of differences in the availability of flowering individuals (Table 1). Only recently opened flowers were sampled and nectar extraction was always carried out on sunny days around noon, although some species can be pollinated during the night.

Nectar sugar concentration in sucrose equivalents [% sugar = (sugar mass/total mass) × 100] was measured with a hand refractometer (Reichert-Jung; range 0–50 %)

only for samples with volumes  $\geq 1 \mu\text{l}$  because of the reading threshold of the refractometer. Nectar sugar composition was analysed using gas–liquid chromatography. Nectar was lyophilized and silylated following Sweeley *et al.* (1963). Derivatives were then injected into a Konik KNK 3000-HRGS gas–liquid chromatograph equipped with a Spectra-Physics SP 4290 data integrator, a flame ionization detector and an OV1 2 m column. Nitrogen was the carrier gas ( $30 \text{ ml min}^{-1}$ ) and the temperature programme used was  $208^\circ\text{C}$  for 1 min,  $1^\circ\text{C min}^{-1}$  until  $215^\circ\text{C}$ ,  $8^\circ\text{C min}^{-1}$  until  $280^\circ\text{C}$ , and maintained for 5 min. Chromatographic sugar analyses were repeated at least twice for each sample in order to control for experimental errors. The sugar ratio ( $r$ ) was calculated as  $r = \text{sucrose}/(\text{fructose} + \text{glucose})$  following Baker and Baker (1983). These authors proposed four sugar ratio categories: sucrose dominant ( $r > 0.999$ ), sucrose rich ( $0.999\text{--}0.5$ ), hexose rich ( $0.499\text{--}0.1$ ) and hexose dominant ( $r < 0.1$ ).

To evaluate the occurrence of relationships between pollinators and nectar characteristics (as suggested by Baker and Baker, 1983), the main flower type for each plant species was determined. Our flower-type concept is relatively similar to the ‘pollination syndrome’ classification (Wyatt, 1983; Proctor *et al.*, 1996), but not only floral characteristics were taken into account but also all information available on their actual associated pollinators or flower visitors. For this purpose, data on flowering phenology, and flower colour, symmetry, depth, and shape were compiled from Brion *et al.* (1988), Correa (1969–1988) and from field observations made by the authors. Information on the flower-visiting fauna of these species was compiled from published records (Riveros, 1991; Ruffini, 1992; Smith-Ramírez, 1993; Forcone *et al.*, 1997; Aizen and Ezcurra, 1998; Bernardello *et al.*, 1999; Aizen *et al.*, 2002; Vázquez and Simberloff, 2003) and unpublished field observations (C. Morales, pers. comm.; M. A. Aizen and V. R. Chalcoff, pers. obs.). Despite using all existing available sources, pollinator information for most plant species should be considered as best educated guesses because of a lack of data on actual measures of pollen transfer. However, it is felt that the present classification into broad categories reflects the main trends in plant–pollinator associations in the temperate forest of South America. According to this, species were classified as hummingbird-pollinated (mostly species with red and tubular corollas), diurnal short-tongued insect-pollinated (mostly species with shallow corollas  $\leq 1 \text{ cm}$  visited by small bees and flies), diurnal long-tongued insect-pollinated (species with corolla tubes  $> 1 \text{ cm}$  but not red, and visited by bumblebees and other large bees) and nocturnal insect-pollinated (species releasing strong odour and/or with nocturnal anthesis and visited by moths and/or sphingids) (Table 2). Only these four flower-type categories were considered because the number of plant species analysed in this study was not large enough to carry out a more detailed classification.

To analyse the effect of plant–pollinator association on nectar sugar concentration and nectar sugar composition, one-way ANOVAs with flower type as the independent variable was used followed by a *posteriori* Tukey test

TABLE 1. The species studied, sample size and reproductive traits of 26 species from the temperate forest of southern South America

Cod	Family/species	P	Locality	Ph	I	F	GF	C	S	D
1	Alstroemeriaceae <i>Alstroemeria aurea</i> Graham	1	Otto Hill	D–F	5	5	H	Y	Z	D
2	Amaryllidaceae <i>Rhodophiala mendocina</i> (Phil.) Rav.	1	Low Chall-huaco Valley	D–J	5	3	H	Y	A	D
3	Asclepiadaceae <i>Cynanchum diemii</i> T. Mey.	1	Lake Escondido	O–N	4	5	V	Y–G	A	S
4	Asteraceae <i>Mutisia decurrens</i> Cav.	1	Low Chall-huaco Valley	D–A	5	5	V	O	A	D
5	<i>Mutisia spinosa</i> Ruiz & Pav.	2	Otto Hill		5	5				
		1	Otto Hill	D–A	5	5	V	L	A	D
		2	Traful		3	5				
6	<i>Perezia prenanthoides</i> Less.	1	Low Chall-huaco Valley	J–F	4	5	H	S	A	S
7	Berberidaceae <i>Berberis buxifolia</i> Lam.	1	Llao–Llao Hill	S–N	1	5	S	Y	A	S
8	<i>Berberis darwinii</i> Hook.	2	Otto Hill		2	5				
		1	Llao–Llao Hill	S–J	2	5	S	O	A	S
		2	Puerto Blest		2	5				
9	Bignoniaceae <i>Campsidium valdivianum</i> (Phil.) Skottsb.	1	Puerto Blest	A–D	5	5	V	R	A	D
10	<i>Eccremocarpus scaber</i> Ruiz & Pav.	1	Lake Traful	O–F	5	5	V	R	Z	D
11	Desfontainiaceae <i>Desfontainia spinosa</i> Ruiz & Pav.	1	Puerto Blest	J–M	5	5	S	R–Y	A	D
12	Elaeocarpaceae <i>Aristotelia chilensis</i> (Molina) Stuntz	1	Otto Hill	N–D	1	5	T	W–R	A	S
13	Escalloniaceae <i>Escallonia rubra</i> (Ruiz & Pav.) Pers.	1	Puerto Blest	D–J	5	5	S	R	A	D
14	Fabaceae <i>Lathyrus multiceps</i> Clos	1	Otto Hill	N–D	5	5	H	S	Z	D
15	<i>Vicia nigricans</i> Hook. & Arn.	1	Low Chall-huaco Valley	O–J	5	5	H	P	Z	D
16	Gesneriaceae <i>Asteranthera ovata</i> (Cav.) Hanst.	1	Puerto Blest	D–A	4	5	E	R	Z	D
17	<i>Mitraria coccinea</i> Cav.	1	Puerto Blest	D–A	5	5	E	O–R	A	D
18	Grossulariaceae <i>Ribes magellanicum</i> Poir.	1	Otto Hill	O–D	4	5	S	Y	A	S
		2	Lake Escondido		1	5				
		3	Llao–Llao Hill		3	5				
19	Loranthaceae <i>Tristerix corymbosus</i> (L.) Kuijt	1	Peninsula San Pedro	M–N	5	5	P	R	A	D
		2	Llao–Llao Forest		5	5				
20	Onagraceae <i>Fuchsia magellanica</i> Lam.	1	Puerto Blest	N–My	5	5	S	R–Pu	A	D
		2	Stream La Virgen		4	5				
21	<i>Oenothera odorata</i> Jacq.	1	237 Route (10 km)	N–A	5	5	H	Y	A	D
22	Philesiaceae <i>Lapageria rosea</i> Ruiz & Pav.	1	Puyehue (Chile)		2	5	V	R	A	D
23	Proteaceae <i>Embothrium coccineum</i> J.R. Forst. & G. Forst.	1	Lake Quillen	O–J	4	5	S	R	Z	D
		2	Traful		5	5				
		3	Piltriquitron Hill		5	5				
		4	Villa la Angostura		5	5				
		5	Otto Hill		5	5				
		6	Puerto Blest		5	5				
		7	Bariloche Airport road		5	5				
		8	Lake Espejo		5	5				
		9	Puyehue (Chile)		5	5				
24	<i>Lomatia ferruginea</i> (Cav.) R. Br.	1	Puerto Blest	D–F	5	5	T	Y–P	Z	S
25	Scrophulariaceae <i>Ourisia poeppigii</i> Benth.	1	Otto Hill	O–J	3	5	H	R	A	D
26	Verbenaceae <i>Diostea juncea</i> (Gillies & hook.) Miers	1	Low Chall-huaco Valley	N–F	5	5	S	S	A	D

Cod, species code number; P, population number; Locality, sampling site; Date, sampling date; Ph, flowering period (J, January; F, February; M, March; A, April; My, May; S, September; O, October; N, November; D, December); I, number of individuals sampled per population; F, number of flowers sampled per individual; GF, growth form (T, tree; S, shrub; E, epiphyte; V, vine; H, herbaceous; P, hemiparasite); C, flower colour (G, green; L, lilac; O, orange; P, pink; Pu, purple; R, red; S, sky-blue; Y, yellow; WR, wine-red); S, flower symmetry (A, actinomorphic; Z, zygomorphic); D, flower depth [D, deep (corolla >1 cm); S, shallow (corolla ≤1 cm)].

TABLE 2. Nectar concentration, sugar proportions, sugar ratio (r) and flower type (FT) of 26 species from the temperate forest of southern South America

Species	P	% Conc.	S	F	G	Uk	r	FT
Alstroemeriaceae								
<i>Alstroemeria aurea</i>	1	40.7	47.3 ± 21.5	18.5 ± 4.2	34.2 ± 19.25	–	0.89	DLTI
Amaryllidaceae								
<i>Rhodophiala mendocina</i>	1	12 ± 2.64	1.4 ± 2.23	38.7 ± 7.21	59.9 ± 7.74	–	0.01	DLTI
Asclepiadaceae								
<i>Cynanchum diemii</i>	1	48.5 ± 2.12	94.9 ± 5.31	2.7 ± 2.96	2.4 ± 2.37	–	18.61	NI
Asteraceae								
<i>Mutisia decurrens</i>	1	41.9 ± 5.48	45.8 ± 13.44	26.5 ± 6.14	27.7 ± 8.94	–	0.85	DLTI
	2	25	54.6 ± 0.01	19.3 ± 0.02	26.1 ± 0.04	–	1.2	
Overall mean		33.5 ± 11.95	50.2 ± 6.22	22.9 ± 5.11	26.9 ± 1.11	–	1.01	
<i>Mutisia spinosa</i>	1	41.3 ± 1.06	71.3 ± 8.92	16.3 ± 5.68	12.4 ± 6.15	–	2.48	DLTI
	2	50.3 ± 5.3	73.1 ± 8.21	19.2 ± 4.22	7.7 ± 3.98	–	2.72	
Overall mean		45.8 ± 6.36	72.1 ± 1.27	17.8 ± 2.03	10.1 ± 3.31	–	2.58	
<i>Perezia prenanthoides</i>	1	42.7 ± 6.52	52 ± 13.6	22.7 ± 8.52	25.3 ± 6.85	–	1.08	DSTI
Berberidaceae								
<i>Berberis buxifolia</i>	1	Nd	58.8 ± 0.26	10.8 ± 0.47	30.4 ± 0.74	–	1.43	DSTI
	2	Nd	80.3 ± 5.54	8.2 ± 6.17	11.5 ± 0.62	–	4.08	
Overall mean			69.6 ± 15.16	9.5 ± 1.78	20.9 ± 13.37	–	2.29	
<i>Berberis darwinii</i>	1	42.5	91 ± 5.7	2 ± 1.97	3.9 ± 4.2	3.1 ± 0.47	15.42	DSTI
	2	30	91.5 ± 3.51	1.2 ± 0.24	2.6 ± 0.44	4.7 ± 2.82	24.08	
Overall mean		36.3 ± 8.83	91.2 ± 0.35	1.6 ± 0.54	3.3 ± 0.96	3.9 ± 1.15	18.61	
Bignoniaceae								
<i>Campsidium valdivianum</i>	1	23.4 ± 2.88	46 ± 10.4	25.4 ± 5.17	28.6 ± 9.6	–	0.85	HUM
<i>Eccremocarpus scaber</i>	1	27 ± 4.32	65.1 ± 11.41	23.4 ± 8.01	11.5 ± 4.03	–	1.87	HUM
Desfontainiaceae								
<i>Desfontainia spinosa</i>	1	20.6 ± 3.41	76.3 ± 10.03	19.4 ± 9.3	4.3 ± 4.77	–	3.22	HUM
Elaeocarpaceae								
<i>Aristotelia chilensis</i>	1	Nd	17.1 ± 0.05	41.4 ± 0.42	41.5 ± 0.37	–	0.2	DSTI
Escalloniaceae								
<i>Escallonia rubra</i>	1	51.7 ± 11.4	26.4 ± 15.84	39.4 ± 8.18	34.2 ± 10.24	–	0.36	HUM
Fabaceae								
<i>Lathyrus multiceps</i>	1	Nd	58.6 ± 5.98	21.8 ± 3.54	19.6 ± 6.74	–	1.42	DLTI
<i>Vicia nigricans</i>	1	29.5 ± 9.25	45.8 ± 28.32	34.4 ± 19.03	19.8 ± 12.29	–	0.84	DLTI
Gesneriaceae								
<i>Asteranthera ovata</i>	1	29.7 ± 5.48	86.4 ± 5.42	10.8 ± 4.82	2 ± 1.49	0.8 ± 1.41	6.75	HUM
<i>Mitraria coccinea</i>	1	30.9 ± 7.43	78.9 ± 8.89	17.3 ± 8.24	2.8 ± 2.2	1 ± 0.71	3.93	HUM
Grossulariaceae								
<i>Ribes magellanicum</i>	1	16 ± 2.82	54.5 ± 8.36	23.3 ± 6.71	22.2 ± 1.66	–	1.2	DSTI
	2	Nd	90.9 ± 1.52	5.8 ± 1	3.3 ± 0.53	–	9.99	
	3	13	57.1 ± 0.52	23.1 ± 0.9	19.8 ± 1.18	–	1.33	
Overall mean		14.5 ± 2.12	67.5 ± 20.3	17.4 ± 10.04	15.1 ± 10.28	–	2.08	
Loranthaceae								
<i>Tristerix corymbosus</i>	1	30.8 ± 6.3	55.3 ± 6.71	19.1 ± 3.13	25.6 ± 4.32	–	1.24	HUM
	2	29.7 ± 5.46	45 ± 7.08	19.6 ± 2.95	35.4 ± 4.13	–	0.82	
Overall mean		30.3 ± 0.77	50.2 ± 7.27	19.4 ± 0.31	30.4 ± 6.95	–	1.01	
Onagraceae								
<i>Fuchsia magellanica</i>	1	23.9 ± 2.74	66 ± 21.61	21.8 ± 15.58	12.2 ± 7.03	–	1.94	HUM
	2	25.3 ± 0.5	65.5 ± 11.17	21 ± 2.41	13.5 ± 8.97	–	1.9	
Overall mean		24.6 ± 0.98	65.8 ± 0.38	21.3 ± 0.55	12.9 ± 0.93	–	1.92	
<i>Oenothera odorata</i>	1	40.7 ± 0.57	95.1 ± 3.58	3 ± 1.74	1.9 ± 1.9	–	19.41	NI
Philesiaceae								
<i>Lapageria rosea</i>	1	27 ± 5.65	91.8 ± 8.11	3 ± 2.53	5.2 ± 5.57	–	11.2	HUM
Proteaceae								
<i>Embothrium coccineum</i>	1	35.3 ± 0.35	67.8 ± 14.87	16.7 ± 6.91	15.5 ± 7.96	–	2.1	HUM–
	2	22.3 ± 1.06	75.6 ± 6.86	10.1 ± 2.96	14.2 ± 3.9	–	3.12	(P)
	3	24 ± 5.56	65 ± 5.29	12.5 ± 6.29	22.5 ± 3.54	–	1.86	
	4	47.4 ± 3.97	97.5 ± 1.44	1.1 ± 0.53	1.4 ± 0.68	–	39	
	5	45.2 ± 2.96	66.1 ± 11.25	15.5 ± 5.54	18.4 ± 6.11	–	1.95	
	6	20.9 ± 10.47	88 ± 7.54	5.7 ± 4.04	6.3 ± 3.6	–	7.33	
	7	21.6 ± 6.42	85.3 ± 3.66	6.4 ± 1.64	8.3 ± 2.24	–	5.8	
	8	42.3 ± 10.51	94.9 ± 2.84	2.1 ± 1.25	3 ± 1.61	–	18.23	
	9	Nd	38.4 ± 12.33	28.3 ± 19.24	33.3 ± 6.9	–	0.62	
Overall mean		29.9 ± 9.79	75.4 ± 18.52	10.9 ± 8.52	13.7 ± 10.22	–	3.06	
<i>Lomatia ferruginea</i>	1	Nd	36 ± 21.98	37.7 ± 14.49	26.3 ± 7.54	–	0.56	DSTI
Scrophulariaceae								
<i>Ourisia poeppigii</i>	1	28	34.9 ± 2.29	29.4 ± 0.66	35.7 ± 1.62	–	0.54	HUM
Verbenaceae								
<i>Diostea juncea</i>	1	34.8 ± 15.21	32.1 ± 18.41	30.2 ± 13	37.7 ± 7.66	–	0.47	DLTI

P, population number (see Table 1); % Conc., nectar concentration (mass/mass as percentage); sugar proportions for S, sucrose; F, fructose; G, glucose; Uk, unknown sugar; r, sugar ratio [S/(F + G)]; FT, flower-type categories (DLTI, diurnal long-tongued insects; DSTI, diurnal short-tongued insects; HUM, hummingbirds; NI, nocturnal insects; P, passerines).

Concentration and sugar percentage values are population means ± standard deviation.

Nd, no data available.

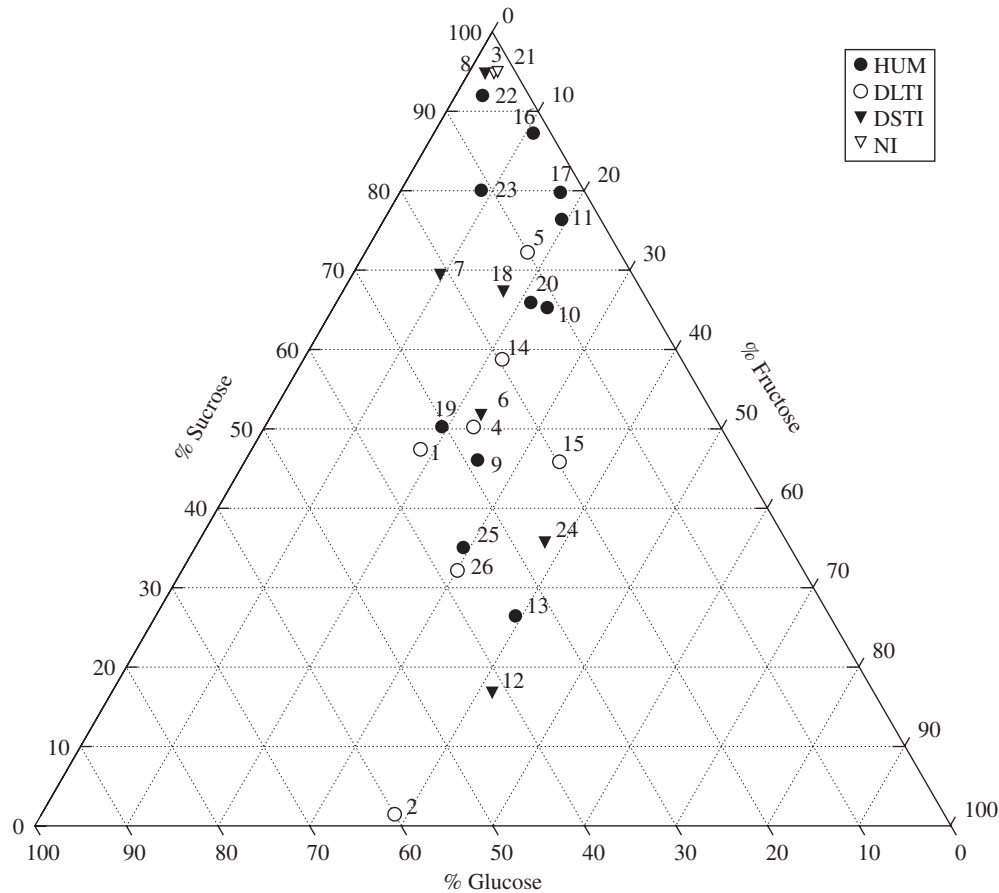


FIG. 1. Ternary diagram of sugar composition for the nectar of 26 species of the temperate forest of southern South America. The numbers refer to the species codes listed in Table 1. Symbols refer to flower-type categories (HUM, hummingbirds; DLTI, diurnal long-tongued insects; DSTI, diurnal short-tongued insects; NI, nocturnal insects. See Table 2).

where appropriate. Because all variables were expressed as percentages or proportions, the arcsin root-square transformation following Sokal and Rohlf (1981) was used.

## RESULTS

### Overall characteristics of nectar

Mean nectar concentration ( $\pm$  standard deviation) for all species was  $31.9 \pm 10.23\%$ , ranging from 12% (*Rhodophiala mendocina*) to 51.7% (*Escallonia rubra*) (Table 2). The three most common sugars (sucrose, glucose and fructose) were found in the nectar of all the 26 species studied. The nectar composition of *Asteranthera ovata*, *Berberis darwinii* and *Mitraria coccinea* included a low percentage (<5%) of an unknown sugar (Table 2). Nectar of 17 of the 26 species (i.e. 65.4%) of the species was sucrose dominant (Table 2 and Fig. 1). It is interesting to see in Fig. 1 that the nectar of most species is distributed non-randomly from the top angle of the compositional triangle to the middle of the bottom axis (about 65%) along the isoline of equal proportions of glucose and fructose.

Striking differences in nectar concentration and composition between species of different genera within the same family for species belonging to contrasting flower types

were observed (Table 2). In the Onagraceae, *Fuchsia magellanica* is hummingbird-pollinated whereas *Oenothera odorata* is nocturnal insect-pollinated, the latter species showing a higher sugar concentration and proportion of sucrose. In addition, in the Proteaceae, *Embothrium coccineum* is hummingbird-pollinated whereas *Lomatia ferruginea* is diurnal short-tongued insect-pollinated, the first species having sucrose-dominant nectar.

The sample used in this study also included pairs of species of the same genus: *Mutisia* (Asteraceae) and *Berberis* (Berberidaceae) (each species is represented by two populations; Table 1). Species from these two genera were classified as diurnal long-tongued insect-pollinated, and diurnal short-tongued insect-pollinated, respectively. Nectar comparisons showed that *M. decurrens* and *B. buxifolia* had a lower proportion of sucrose than nectars of *M. spinosa* and *B. darwinii* (Table 2). In *Berberis darwinii*, a fourth unknown sugar that was not present in *B. buxifolia* was also detected (Table 2).

Intraspecific variation in nectar traits was evaluated in those species with two or more populations sampled (six species were represented by two populations, *Ribes magellanicum* by three populations, and *Embothrium coccineum* by nine populations; Table 1). Particularly, large interpopulation variability (i.e. CVs >50%) in sugar composition was

TABLE 3. Coefficient of variation [ $CV = (SD/species\ mean) \times 100$ ; data are from populations] for sugar concentration (% Conc.) and nectar proportion of sucrose (S), fructose (F) and glucose (G) for the eight species with more than one population studied

	N pop.	% Con	S	F	G
<i>Mutisia decurrens</i>	2	35.7	12.4	22.3	4.1
<i>Mutisia spinosa</i>	2	13.9	1.7	11.4	32.8
<i>Berberis buxifolia</i>	2	Nd	21.7	18.7	<b>63.9</b>
<i>Berberis darwinii</i>	2	24.3	0.38	33.7	29.1
<i>Ribes magellanicum</i>	3	14.6	30.1	<b>57.7</b>	<b>68.1</b>
<i>Tristerix corymbosus</i>	2	2.5	14.5	1.6	22.8
<i>Fuchsia magellanica</i>	2	3.9	0.58	2.6	7.2
<i>Embothrium coccineum</i>	9	32.7	24.6	<b>78.2</b>	<b>74.6</b>

N pop., number of populations (see Table 1).  
The values in bold correspond to CVs >50 %.  
Nd, no data available.

observed for *B. darwinii*, *R. magellanicum* and *E. coccineum* (Table 3).

#### Nectar traits and flower types

Nectar concentration did not differ significantly among flower-type categories (one-way ANOVA,  $F_{3,18} = 1.19$ ,  $P = 0.34$ ). Nevertheless, mean nectar concentration was comparatively lower for hummingbird-pollinated species ( $27.0 \pm 3.6\%$ ), intermediate for diurnal long-tongued and diurnal short-tongued insect-pollinated species ( $32.7 \pm 11.7\%$  and  $31.2 \pm 14.8\%$ , respectively), and higher for nocturnal insect-pollinated species ( $44.6 \pm 5.5\%$ ) (Table 4).

Hummingbird- and nocturnal insect-pollinated species showed higher sucrose proportions than diurnal short- and long-tongued insect-pollinated species (Table 4 and Fig. 1). Hummingbird-, diurnal short-tongued insect- and long-tongued insect-pollinated species showed a mean nectar sugar composition with comparable variabilities (Table 4). On the other hand, nocturnal insect-pollinated species showed lower variability for nectar sugar composition as well as for nectar concentration, although a low number of species was sampled in this group (Table 4). Significant differences were found among flower-type groups for the sugar ratio ( $r$ ) ( $F_{3,22} = 7.66$ ,  $P = 0.001$ ), and sucrose percentage ( $F_{3,22} = 3.3$ ,  $P = 0.039$ ), but *a posteriori* tests showed that these differences could be attributed to the high sucrose content of the nocturnal insect-pollinated species in comparison with the other three remaining groups. In spite of this, the differences are basically among the nocturnal insect-pollinated group (sugar ratio of 19 and sucrose proportion of 95 %) and the diurnal long-tongued insect-pollinated group (sugar ratio of 1.03 and sucrose proportion of 43 %).

## DISCUSSION

In general terms, the present results showed that average nectar concentration for species of the temperate forest of southern South America was low, particularly in comparison to the average nectar concentration found among

species from different sites of the neighbouring Patagonian steppe [31.9 % for temperate forest in comparison to 44.6 and 42.4 % for steppe sites; authors' data, Forcone *et al.* (1997) and Bernardello *et al.* (1999), respectively]. This trend could be related to the large proportion of hummingbird-pollinated species of the temperate forest of southern South America, reflecting the higher incidence of ornithophily in this region compared with the steppe (Aizen and Ezcurra, 1998). The deep corolla tubes characteristic of hummingbird-pollinated flowers are usually associated with nectars of low concentration due to either lower evaporation than in more open flowers (Plowright, 1987) or the innate preferences, physical limitations and special requirements of hummingbirds for sugar solutions of low viscosity (Pyke and Waser, 1981, and references therein). However, when hummingbird-pollinated species were excluded to estimate the mean sugar concentration for insect-pollinated plant species of the temperate forest, a comparatively low nectar concentration ( $34.45 \pm 11.85\%$ ) was still obtained.

Nectar concentration is highly influenced by environmental factors, especially temperature and humidity (Rathcke, 1992). The differences in mean nectar concentration between forest plants and those from the Patagonian steppe (Forcone *et al.*, 1997; Bernardello *et al.*, 1999) can also be explained when considering the contrasting environmental particularities of these two regions. The relatively low mean nectar concentration of temperate forest species could be related to the lower mean maximum temperatures and higher precipitation characteristic of the forest environment in comparison with the nearby steppe (Barros *et al.*, 1983).

In general terms, the present results show that nectar composition of species from the temperate forest of southern South America is mainly sucrose dominant. Only a low number of plant species had hexose-dominant (only *Rodophiala mendocina*) or hexose-rich nectars (*Aristotelia chilensis*, *Escallonia rubra* and *Diostea juncea*). This trend is again in contrast with the Patagonian steppe where nectars are mainly hexose rich or hexose dominant (Forcone *et al.*, 1997; Bernardello *et al.*, 1999).

Plant species of the temperate forest of southern South America did not show a clear-cut association between nectar concentration, sugar composition and pollinators. However, some weak trends resulted from the present analysis. For example, hummingbird- and nocturnal insect-pollinated species secrete nectars dominated by sucrose, whereas diurnal long- and short-tongued insect-pollinated flowers tend to produce nectar with a similar proportion between sucrose and hexoses (i.e. sucrose-hexose balanced nectars), suggesting a convergence in sugar composition of some species from different families according to the pollinators. Hummingbird flowers have been reported elsewhere to produce nectars with a relatively high proportion of sucrose (e.g. Cruden *et al.*, 1983; Freeman *et al.*, 1984; Gottsberger *et al.*, 1984; Freeman and Worthington, 1985; Elisens and Freeman, 1988; Stiles and Freeman, 1993) and, in general, these birds prefer sucrose- over hexose-rich solutions (Hainsworth and Wolf, 1976; Stiles, 1976; Martínez del Rio, 1990). In the case of *Sephanoides sephanoides*, the preference of this species for sucrose-rich solutions

TABLE 4. Mean nectar concentration [% Conc.: (sugar mass/total mass) × 100], sugar proportions (S, sucrose; F, fructose; G, glucose), and sugar ratio [r, S/(F+G)] for the different flower type categories (DSTI, diurnal short-tongued insect-pollinated; DLTI, diurnal long-tongued insect-pollinated; HUM, hummingbird-pollinated; NI, nocturnal insect-pollinated)

	<i>n</i>	% Conc.	S	F	G	<i>r</i>
DSTI	6	31.16 ± 14.78	56.18 ± 27.42	21.73 ± 15.57	22.08 ± 12.68	4.14 ± 7.14
DLTI	7	32.72 ± 11.66	43.93 ± 22.4	26.33 ± 8.16	29.74 ± 16.27	1.03 ± 0.81
HUM	11	27.02 ± 3.59	63.93 ± 21.76	19.82 ± 10.04	16.26 ± 13.27	3.24 ± 3.26
NI	2	44.60 ± 5.52	95.00 ± 0.14	2.80 ± 0.21	02.20 ± 0.35	19.0 ± 0.56

Values are group means ± standard deviation.  
*n* = number of species in each category group.

was experimentally corroborated by field experiments (Chalcoff, 2001).

It is interesting to point out that *Ourisia poeppigii*, a species with red tubular flowers but without field records of hummingbird visits (it was considered as a hummingbird-pollinated species based solely on the analysis of floral traits), showed the lowest relative proportion of sucrose among the sample of species with tubular red flowers from the temperate forest of South America. In addition, few records of hummingbird visits have been reported (Fraga *et al.*, 1997) for *Escallonia rubra*, a species with a comparatively high nectar concentration with a low percentage of sucrose. Nevertheless, when these species are excluded from the analysis, the trends observed did not change. In addition, *Ourisia poeppigii* apparently has a high fruit and seed set, which suggests a highly autogamous breeding system, despite producing showy flowers (M. A. Aizen, pers. obs.).

In general, nectar traits were shown to be highly variable at any taxonomic scale. This is the case for the two species pairs analysed that were from different genera but belonging to the same family (e.g. Onagraceae and Proteaceae). Each member of these pairs of species is characterized by a particular nectar composition that seems to relate to its association with different pollinators. Nevertheless, pairs of congeneric species (*Mutisia* and *Berberis*) present divergent sugar ratios despite their association with similar pollinator assemblages (Table 2).

Variation at the intraspecific level can be exemplified by the study of nectar traits in nine populations of *E. coccineum*. This species, a self-incompatible endemic tree of this forest, seems to have divergent bird-pollinator assemblages on the Chilean and Argentine side of the Andes (Fraga *et al.*, 1997; Smith-Ramírez and Armesto, 1998). Accordingly, the present results show a divergent nectar composition pattern, which agrees with reported differences in nectar preferences by their two main pollinator types. The Chilean populations are reported as passerine-pollinated and with nectars dominated by hexoses (Smith-Ramírez and Armesto, 1998; M. Riveros, pers. comm.), and the nectar of the only Chilean population reported here is also dominated by hexoses. In contrast, the Argentine populations are mainly hummingbird-pollinated (Fraga *et al.*, 1997; V. R. Chalcoff, pers. obs.) and their nectars are dominated by sucrose (Bernardello *et al.*, 1999; and this study). In addition to hummingbirds, some diurnal insects have

been reported to be pollinators of this species in Argentina, at the northern range of its distribution (Devoto *et al.*, 2006). There is no actual evidence of flower visits by passerines in the Argentine populations, despite their high abundance during spring and summer (Amico and Aizen, 2005; M. A. Aizen, pers. obs.). Thus, nectar-sugar composition in this species seems to be closely related to different bird-pollinator assemblages on both sides of the Andes, and at least part of the regional variation in the nectar sugar composition could be explained by divergent selection mediated by these birds. However, a large amount of variation was still found in nectar traits among Argentine populations of *E. coccineum* that could be related to environmental plasticity, local selective factors and genetic drift. This large intraspecific variation in nectar sugar composition shows the underlying potential for evolutionary change in nectar traits under different selective pressures (cf. Schluter, 2000), a situation that merits further investigation.

Nevertheless, most of the present results showed that pollinators are not the only force modelling nectar traits, particularly sugar composition, in the flora of the temperate forest of southern South America. Other authors have pointed out a more complex evolutionary scenario which includes interactions with herbivores, nectar robbers, seed predators and/or seed dispersers influencing different reproductive traits in plants (e.g. Armbruster, 1997; Galen, 1999; Herrera, 2000; Aizen, 2003; Irwin *et al.*, 2004). In the case of the flora of the temperate forest of southern South America, it is felt that its complex and ancient biogeographical history and its current development and occurrence on striking environmental gradients (Aizen and Ezcurra, 1998) may be important in understanding present patterns in nectar characteristics.

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