

Are Nectar Sugar Composition and Corolla Tube Length Related to the Diversity of Insects that Visit Asteraceae Flowers?

C. Torres and L. Galetto

Instituto Multidisciplinario de Biología Vegetal (Universidad Nacional de Córdoba-CONICET), Córdoba, Argentina

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Abstract: In this work, we analysed interspecific variation in nectar sugar composition, corolla tube length, and the diversity of floral visitors of 35 Asteraceae species. The potential correlations between these variables could arise either as a result of selection to improve pollinator attractiveness or simply as a consequence of phylogenetic constraints. Samples of nectar and flowers, and data on floral visitors, were obtained from living plants in natural populations from Argentina. Asteraceae species showed a large variability in corolla tube length. Nectar of most species presented a larger proportion of hexoses than sucrose. All species were visited by numerous insects belonging to ≥ 2 different orders. Results showed that floral traits are not significantly correlated with the diversity of floral visitors. These characters seem to be linked to the phylogeny of the species. Early branching species (species phylogenetically close to the root of the Asteraceae tree) tend to have longer corollas, higher sucrose proportions and lesser diversity of floral visitors than late branching species. Considering that longer corolla tubes and higher nectar sucrose percentages may indicate some specialization in the pollination system, we suggest that there is an evolutionary tendency toward generalist pollination systems within the family.

Key words: Compositae, generalization, nectar, corolla depth, phylogenetic constraints, floral visitors, pollination.

Introduction

Pollinators must make economic decisions when faced with a diverse array of flowers of varying structure, colour and reward. The amount of nectar sucrose and the corolla tube length are two floral traits that have been frequently related to pollinator foraging choices (e.g., Dafni and Neal, 1997^[10]; Galetto et al., 2000^[18] and references therein). When a group of similarly pollinated species shows a floral trait with far less variability than encountered in more random samples, flower specialization must be suspected, because a trait under directional selection can experience a reduction in phenotypic variation (Fenster, 1991^[13]). However, several authors have point-

ed out that members of a single clade can be expected to have the same floral trait because they share recent ancestors, rather than because they share some ecological feature (Armbruster, 1996^[1]; Silvertown and Dodd, 1997^[38]).

Floral nectar is the most important reward offered to potential pollinators in the angiosperms as a whole (Simpson and Neff, 1983^[39]). Although nectar contains a wide variety of chemicals, three common sugars – fructose, glucose and sucrose – dominate the total solutes (e.g., Percival, 1961^[33]; Baker and Baker, 1983a^[3]; Gottsberger et al., 1984^[18]; Galetto, 1993^[16]; Stiles and Freeman, 1993^[45]). The relative concentrations of these sugars can show wide interspecific variation and have been related to the pollinator guild or to the flower syndrome of the plant species (e.g., Baker and Baker, 1990^[5]). The differences of pollinator preferences have been explained on the basis of i) the specific taste and odour that different sugars impart to nectar (e.g., Stiles, 1976^[44]; Pham-Delegue et al., 1990^[35]; Southwick, 1990^[42]), ii) the enzymatic capability of each floral visitor related to the efficiency of sucrose absorption (Martínez del Río et al., 1988^[29]; Downs and Perrin, 1996^[11]) and iii) the different sugar compositions needed to fulfill nutritional and energetic requirements of animals (Heinrich and Raven, 1972^[23]; Southwick et al., 1981^[41]).

However, caution must be assumed when interpreting nectar characteristics strictly in terms of pollinator selection. There is a tendency for long-tubed flowers to present sucrose-dominated nectars, while hexose-dominated nectars tend to be associated with short-tubed flowers (Southwick et al., 1981^[41] and references therein; Freeman and Worthington, 1985^[14]; Morales, 1999^[31]). Therefore, corolla tube length seems to be correlated to sugar composition and hence, it could be indirectly determining plant's attractiveness and pollinator choices.

Flower depth can also directly affect pollinator foraging strategies independently of nectar sugar composition. Since efficient nectar extraction requires a proboscis that roughly matches the length of the corolla tube (e.g., Goulson, 1999^[20]), an increase in the corolla tube length may be accompanied by a reduction in the diversity of pollinators (Fenster, 1991^[13]; Plowright and Plowright, 1997^[36] but see McCall and Primack, 1992^[30]; Herrera, 1996^[24]). Thus, long corolla tubes may allow species to protect their nectar from generalist pollinators (Heinrich, 1983^[22]).

In consequence, regarding the antecedents about the interrelationships between the nectar sugar types, pollinator preferences and flower depth, it can be assumed that longer corolla tubes and higher nectar sucrose percentages are traits that may indicate some specialization in the pollinator guild (e.g., Baker and Baker, 1990^[5]; Goulson, 1999^[20]). Thus, we can expect that taxa with generalist pollination systems have lesser quantities of nectar sucrose and shorter corolla tubes than taxa with specialized pollination.

Although Asteraceae species are a significant component of almost all terrestrial ecosystems, the pollination biology of relatively few taxa has been examined in detail (Lane, 1996^[27]). This family is particularly poorly known in terms of their nectar constituents, considering that, of over 23000 species of Asteraceae (Judd et al., 1999^[25]), only 75 species have been studied in relation to their nectar sugar composition (Wykes, 1952^[52]; Percival, 1961^[33]; Van Handel et al., 1972^[50]; Hainsworth and Wolf, 1976^[21]; Kapila, 1978^[26]; Neff and Simpson, 1990^[32]; Pham-Delegue et al., 1990^[35]; Bernardello et al., 1994^[7], 1999^[8]; Galetto, 1995^[17]; Baker et al., 1998^[6]; Torres, 1998^[49]).

In this work, we analyse interspecific variation in nectar sugar composition, corolla tube length and the diversity of floral visitors to 35 Asteraceae species. In addition, we also consider the possible relationships between these variables and flower colours. The potential correlations between these variables could arise either as a result of selection to improve pollinator attractiveness or simply as a consequence of phylogenetic constraints.

Materials and Methods

Samples of nectar and flowers, and data on floral visitors, were obtained from living plants in natural populations from Argentina, Cordoba Province, Dept. Santa Marıa: Los Aromos (except for *Vernonia fulva* which was studied in Tucuman Province). Voucher specimens are deposited in the herbarium of the Museo Botanico de Cordoba (CORD).

Nectar was extracted with glass capillary tubes and preserved at -18°C . Sugar separation was accomplished by gas chromatography. Nectar was lyophilised and silylated according to Sweeley et al. (1963^[46]). The derivatives were then injected into a Konik KNK 3000-HRGS gas chromatograph equipped with a Spectra-Physics SP 4290 data integrator, a flame ionization detector, and an OV 101 3% column (2 m long) on Chromosorb G/AW-DMCS mesh 100–120. Nitrogen was the carrier gas (30 ml min^{-1}) and the following temperature programme was used: 208°C for 1 min, $1^{\circ}\text{C min}^{-1}$ until 215°C , $10^{\circ}\text{C min}^{-1}$ until 280°C for 2 min. Carbohydrate standards (Sigma Chem.) were prepared using the same method. Chromatographic sugar analyses were run at least twice for each sample. Sucrose ratio (Sr) and hexose ratio (Hr) were calculated as follows: $\text{Sr} = \text{sucrose} / (\text{fructose} + \text{glucose})$ and $\text{Hr} = \text{glucose} / \text{fructose}$.

Capitula were preserved in 70% ethanol. To estimate mean corolla tube length for each species, the corolla of one flower from each of five individuals were measured with a digital caliper (resolution = 0.01 mm) and the assistance of a Zeiss Stemi SV 6 stereoscopic microscope. The corolla tube length was considered as the distance between corolla insertion and the be-

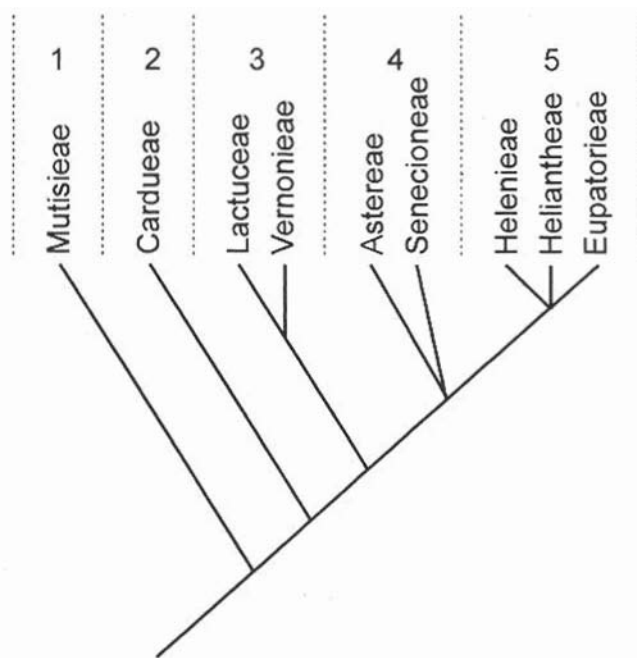


Fig. 1 Diagram of phylogenetic interrelationships of the tribes of the Asteraceae species studied, adapted from Bremer, 1996^[9]. The five monophyletic groups are indicated by dotted lines.

ginning of corolla lobes. In the capitula with different morphological flower types and in dioecious species, corolla measurements were performed only on hermaphrodite or female flowers. In general, Asteraceae species showed a high frequency of flower visitors during exploratory 10-min observations. Thus, we considered that 2 h of observations for each species were sufficient time to obtain a reasonable measure of visitor diversity. Observations on each species were made in four periods of 30 min, equally distributed in the morning and afternoon of different sampling days.

Data were subjected to correlation analyses (Pearson coefficient and Spearman coefficient for Sr). In order to remove the lack of independence that common ancestry may confer on samples (Silvertown and Dodd, 1997^[38]), additional correlation tests were made, considering monophyletic groups of species as the statistical units. Although there is a large amount of work on phylogenetic reconstruction of the Asteraceae family, there are still problems in general and basal resolution of many tribes. In this way, although data concern species belonging to nine tribes, only five monophyletic groups of species were considered (Fig. 1), according to the phylogenetic diagram proposed by Bremer (1996^[9]).

We used hierarchical (nested) statistical methods to partition the total variance and covariance of floral traits into different phylogenetic organizational levels. Since, in general, there was not a substantial correlation between the dependent variables and covariates, the increase of the test sensitivity of analysis of covariance (due to a reduction in the error variance) did not offset the loss of a degree of freedom for the error (Sokal and Rohlf, 1995^[40]; Tabachnick and Fidell, 1996^[47]). Thus, we have only considered nested analyses of variance (ANOVAs) results. Data were organized into five monophyletic subgroups

Table 1 Nectar sugar composition of 35 Asteraceae species. Values are means \pm SD. Species are arranged according to the phylogenetic tree proposed by Bremer, 1996^[9] (Fig. 1). Abbreviations: \bar{x} = mean of means; Sr = sugar ratio: sucrose/fructose + glucose; Hr = hexose ratio: glucose/fructose; nd = no data

Subfamily	Tribe	Species	Sample	Fructose (%)	Glucose (%)	Sucrose (%)	Unidentified	Sr	Hr
Carduoideae	Mutisieae	<i>Trichocline reptans</i>	1	40.89 \pm 0.30	57.97 \pm 0.08	1.13 \pm 0.22	0	0.01 \pm 0.002	1.43 \pm 0.01
			2	46.96 \pm 0.12	51.93 \pm 0.13	1.1 \pm 0.25	0	0.01 \pm 0.002	1.11 \pm 0.01
			\bar{x}	43.92 \pm 4.29	54.95 \pm 4.27	1.11 \pm 0.02	0	0.01	1.25
	Cardueae	<i>Trixis divaricata</i> var. <i>discolor</i>		32.67 \pm 0.58	44.13 \pm 0.60	23.19 \pm 1.18	0	0.30 \pm 0.02	1.35 \pm 0.01
				30.14 \pm 3.28	31.59 \pm 0.72	40.58 \pm 0.06	0	0.68 \pm 0.01	1.14 \pm 0.06
	Lactuceae	<i>Carduus thoermeri</i>		27.81 \pm 0.80	52.81 \pm 0.30	0	0	0	1.12 \pm 0.01
			1	47.18 \pm 0.30	52.81 \pm 0.30	0	0	0	1.28 \pm 0.12
			2	43.84 \pm 2.27	56.16 \pm 2.27	0	0	0	1.20
		<i>Cichorium intybus</i>	\bar{x}	45.51 \pm 2.36	54.48 \pm 2.37	0	0	0	1.20
				35.29 \pm 1.96	60.23 \pm 2.15	4.47 \pm 0.19	0	0.05 \pm 0.01	1.71 \pm 0.16
Vernonieae		<i>Hypochaeris radicata</i>		30.14 \pm 3.28	61.03 \pm 0.61	8.82 \pm 3.90	0	0.10 \pm 0.05	2.02 \pm 0.20
				19.93 \pm 17.53	29.01 \pm 19.86	50.79 \pm 37.26	0	1.04 \pm 0.05	1.45 \pm 0.20
				36.03 \pm 0.42	51.60 \pm 0.49	12.27 \pm 0.21	0.09 \pm 0.13	0.14 \pm 0.01	1.43 \pm 0.03
		<i>Vernonia fulva</i>	1	27.54 \pm 0.92	41.63 \pm 1.34	30.81 \pm 0.42	0	0.44 \pm 0.01	1.51 \pm 0.10
			2	31.78 \pm 6.01	46.61 \pm 7.05	21.54 \pm 13.11	0.04 \pm 0.06	0.27	1.47
			\bar{x}	9.76 \pm 0.30	14.69 \pm 0.78	75.54 \pm 0.47	0	3.09 \pm 0.08	1.51 \pm 0.12
		<i>V. nudiflora</i>	1	8.01 \pm 0.89	17.65 \pm 3.23	74.32 \pm 4.12	0	2.95 \pm 0.63	2.19 \pm 0.16
			2	8.88 \pm 1.24	16.17 \pm 2.09	74.93 \pm 0.86	0	2.99	1.82
			\bar{x}	38.76 \pm 0.48	59.87 \pm 0.52	1.24 \pm 0.21	0.12 \pm 0.21	0.01 \pm 0.01	1.54 \pm 0.03
		<i>Grindelia discoidea</i>		36.75 \pm 0.18	45.12 \pm 0.64	18.12 \pm 0.81	0	0.22 \pm 0.01	1.23 \pm 0.01
Asteroideae	Astereae	<i>Solidago chilensis</i>		34.53 \pm 1.09	64.17 \pm 0.25	1.3 \pm 0.83	0	0.01 \pm 0.01	1.86 \pm 0.07
				44.42 \pm 3.44	55.57 \pm 3.44	0	0	0	1.26 \pm 0.17
			1	41.14 \pm 1.31	58.28 \pm 1.51	0	0.58 \pm 1.01	0	1.42 \pm 0.08
	Senecioneae	<i>Senecio pampeanus</i>	2	43.03 \pm 0.92	56.96 \pm 0.91	0	0	0	1.32 \pm 0.05
			3	27.91 \pm 1.74	72.09 \pm 1.74	0	0	0	2.59 \pm 0.22
			4	39.12 \pm 7.58	60.72 \pm 7.66	0	0.14 \pm 0.29	0	1.55
	Helenieae	<i>Gaillardia megapotamica</i>	\bar{x}	41.25 \pm 0.03	58.75 \pm 0.03	0	0	0	1.42 \pm 0.01
			1	43.25 \pm 1.76	55.50 \pm 1.42	1.24 \pm 0.34	0	0.01 \pm 0.01	1.28 \pm 0.08
			2	42.25 \pm 1.41	57.12 \pm 2.30	0.62 \pm 0.88	0	0.01	1.35
	Heliantheae	<i>Tagetes minuta</i>		29.83 \pm 1.31	59.41 \pm 0.80	10.75 \pm 0.33	0	0.12 \pm 0.01	1.99 \pm 0.10
				36.01 \pm 4.31	51.07 \pm 0.68	12.91 \pm 3.63	0	0.15 \pm 0.05	1.43 \pm 0.19
				7.41 \pm 1.24	3.15 \pm 0.17	89.43 \pm 1.07	0	8.51 \pm 0.96	0.43 \pm 0.09
		<i>Acanthospermum hispidum</i>		29.11 \pm 0.28	48.34 \pm 5.09	22.55 \pm 4.81	0	0.29 \pm 0.08	1.66 \pm 0.19
				50.23 \pm 2.93	44.66 \pm 2.39	4.07 \pm 0.37	1.01 \pm 0.15	0.04 \pm 0.01	0.89 \pm 0.10
				23.84 \pm 0.94	24.02 \pm 0.52	52.13 \pm 0.41	0	1.09 \pm 0.02	1.01 \pm 0.06
		<i>Acemella decumbens</i> var. <i>affinis</i>	1	41.68 \pm 0.16	39.07 \pm 1.12	19.24 \pm 1.28	0	0.24 \pm 0.02	0.94 \pm 0.02
			2	32.76 \pm 12.61	31.54 \pm 10.64	35.68 \pm 23.26	0	0.55	0.96
			\bar{x}	43.02 \pm 1.02	51.77 \pm 1.09	5.21 \pm 0.07	0	0.05 \pm 0.01	1.2 \pm 0.05
		<i>Angelophytum aspilioides</i>	1	40.82 \pm 0.43	49.95 \pm 0.80	9.22 \pm 0.37	0	0.10 \pm 0.01	1.22 \pm 0.03
Eupatorieae		<i>Bidens andicola</i> var. <i>decomposita</i>	2	41.92 \pm 1.55	50.86 \pm 1.29	7.21 \pm 2.83	0	0.08	1.21
				46.95 \pm 0.25	53.05 \pm 0.25	0	0	0	1.13 \pm 0.01
				41.76 \pm 0.87	53.69 \pm 1.04	4.53 \pm 0.16	0	0.05 \pm 0.01	1.29 \pm 0.05
		<i>R. laevis</i>	1	39.62 \pm 1.26	48.58 \pm 2.74	10.65 \pm 2.88	1.15 \pm 0.93	0.12 \pm 0.03	1.23 \pm 0.10
			2	40.69 \pm 1.51	51.13 \pm 3.61	7.59 \pm 4.33	0.57 \pm 0.81	0.08	1.26
			\bar{x}	32.71 \pm 0.13	67.28 \pm 0.13	0	0	0	2.06 \pm 0.01
		<i>B. pilosa</i>		25.05 \pm 1.67	54.25 \pm 0.07	20.47 \pm 1.41	0	0.26 \pm 0.02	2.17 \pm 0.14
				47.66 \pm 1.60	51.84 \pm 1.72	0	0.49 \pm 0.11	0	1.09 \pm 0.07
			1	46.55 \pm 0.88	40.77 \pm 0.88	12.68 \pm 1.75	0	0.14 \pm 0.02	0.87 \pm 0.01
		<i>Cosmos sulphureus</i>	2	45.47 \pm 0.84	54.52 \pm 0.84	0	0	0	1.20 \pm 0.04
			\bar{x}	46.56 \pm 1.09	49.04 \pm 7.29	4.23 \pm 7.32	0.16 \pm 0.28	0.04	1.05
Eupatorieae		<i>Flourensia campestris</i>		2.33 \pm 0.58	7.33 \pm 1.15	90.33 \pm 0.58	0	9.37 \pm 0.64	3.33 \pm 1.15
				18.45 \pm 0.56	25.35 \pm 1.59	56.19 \pm 1.03	0	1.28 \pm 0.05	1.38 \pm 0.13
				40.01 \pm 0.81	59.99 \pm 0.82	0	0	0	1.50 \pm 0.05
		<i>Eupatorium amottianum</i>	1	46.38 \pm 0.47	49.60 \pm 0.66	4.01 \pm 0.18	0	0.04 \pm 0.01	1.07 \pm 0.02
			2	35.42 \pm 1.46	50.51 \pm 4.81	12.06 \pm 4.64	0	0.17 \pm 0.06	1.43 \pm 0.16
			\bar{x}	40.9 \pm 7.75	50.05 \pm 0.64	8.03 \pm 5.69	0	0.09	1.22
		<i>E. hookerianum</i>		40.06 \pm 0.60	44.02 \pm 1.72	15.91 \pm 2.26	0	0.19 \pm 0.03	1.10 \pm 0.03
				40.54 \pm 0.10	22.27 \pm 0.18	37.18 \pm 0.28	0	0.59 \pm 7.17	0.55 \pm 3.19
				56.53 \pm 1.09	43.47 \pm 1.09	0	0	0	0.77 \pm 0.03
		<i>Mikania periplocaifolia</i>		47.01 \pm 4.66	43.57 \pm 8.51	9.42 \pm 5.82	0	0.10 \pm 0.01	0.93 \pm 0.18
				50.39 \pm 0.73	49.60 \pm 0.73	0	0	0	0.98 \pm 0.03
Eupatorieae		<i>M. urticifolia</i>	1	34.03 \pm 2.08	58.37 \pm 2.49	7.59 \pm 0.41	0	0.08 \pm 0.01	1.72 \pm 0.18
			2	42.21 \pm 11.57	53.98 \pm 6.20	3.79 \pm 5.37	0	0.04	1.28
			\bar{x}						
		<i>Stevia satoreifolia</i>							
		<i>E. subastatum</i>							
		<i>E. viscidum</i>							

Table 2 Flower colour, corolla tube length and floral visitors of 35 Asteraceae species. Floral visitor data show the number of species per insect order. Taxa were arranged according to the phylogenetic tree proposed by Bremer, 1996^[9] (Fig. 1). Abbreviations: nd = no data

Subfamily	Tribe	Species	Flower colour	Corolla tube length (mm) mean \pm S.D.	Hymenoptera	Diptera	Lepidoptera	Coleoptera	Total # of species
	Mutisieae	<i>Trichopline reptans</i>	yellow	10.32 \pm 0.47	4	1	2	0	7
		<i>Trixis divaricata</i> var. <i>discolor</i>	yellow	5.39 \pm 0.59	4	1	0	0	5
Carduoideae	Cardueae	<i>Carduus thoermeri</i>	purple	19.71 \pm 2.36	6	1	3	1	11
Cichorioideae	Lactuceae	<i>Cichorium intybus</i>	sky blue	6.33 \pm 0.24	6	0	0	0	6
		<i>Hypochoeris radicata</i>	yellow	11.45 \pm 1.08	2	1	0	0	3
		<i>Sonchus oleraceus</i>	yellow	10.83 \pm 1.77	3	5	0	0	8
	Vernonieae	<i>Taraxacum officinale</i>	yellow	10.94 \pm 0.95	3	3	2	0	8
		<i>Vernonia fulva</i>	purple	9.85 \pm 0.41	nd	nd	nd	nd	nd
		<i>V. mollissima</i>	purple	8.28 \pm 0.37	9	0	3	0	12
	Asteraceae	<i>V. nudiflora</i>	purple	7.30 \pm 0.66	6	0	2	0	8
	Astereae	<i>Baccharis articulata</i>	white	2.25 \pm 0.36	5	7	4	7	23
		<i>B. rufescens</i>	white	3.91 \pm 0.47	4	1	3	1	9
	Senecioneae	<i>Grindelia discoidea</i>	yellow	5.88 \pm 0.59	6	0	1	1	8
		<i>Solidago chilensis</i>	yellow	3.49 \pm 0.40	8	5	6	1	20
		<i>Senecio pampeanus</i>	yellow	6.18 \pm 0.79	9	9	6	4	28
	Helenieae	<i>Gaillardia megapotamica</i>	yellow	5.73 \pm 0.20	5	2	2	2	11
		<i>Helenium argentinum</i>	yellow	4.67 \pm 0.25	6	3	2	1	12
		<i>Tagetes minuta</i>	yellow	4.12 \pm 1.14	nd	nd	nd	nd	nd
	Heliantheae	<i>Acanthospermum hispidum</i>	white	1.36 \pm 0.20	nd	nd	nd	nd	nd
		<i>Acmella decumbens</i> var. <i>affinis</i>	yellow	2.42 \pm 0.14	2	6	2	6	16
		<i>Angelphytum aspilioides</i>	yellow	4.58 \pm 0.31	5	3	4	1	13
		<i>Bidens laevis</i>	yellow	3.88 \pm 0.28	nd	nd	nd	nd	nd
		<i>B. pilosa</i>	yellow	4.76 \pm 0.85	12	7	6	0	25
		<i>Cosmos sulphureus</i>	orange	7.24 \pm 1.65	6	3	4	0	13
		<i>Flourensia campestris</i>	yellow	4.25 \pm 0.42	8	2	1	1	12
		<i>Pascalina glauca</i>	yellow	5.64 \pm 0.12	nd	nd	nd	nd	nd
		<i>Wedelia buphtalmiflora</i>	yellow	6.93 \pm 0.49	6	2	3	1	12
		<i>Zinnia peruviana</i>	red	6.34 \pm 0.61	1	0	2	0	3
	Eupatorieae	<i>Eupatorium argentinum</i>	lilac	3.25 \pm 0.41	2	1	0	2	5
		<i>E. arnottianum</i>	lilac	5.20 \pm 0.30	3	0	2	1	6
		<i>E. inulaefolium</i>	white	3.90 \pm 0.16	3	4	10	3	20
		<i>E. hookerianum</i>	lilac	5.10 \pm 0.14	3	0	4	0	7
		<i>E. subhastatum</i>	lilac	5.14 \pm 0.55	nd	nd	nd	nd	nd
		<i>Mikania urticifolia</i>	white	7.96 \pm 0.48	10	4	6	3	23
		<i>Stevia satpureifolia</i>	pink	7.24 \pm 0.50	1	1	2	0	4

of tribes (see Fig. 1), nested within two larger groups representing early branching species (taxa phylogenetically close to the root of the Asteraceae tree) and late branching species (early group = subgroups 1, 2 and 3; late group = subgroups 4 and 5; Fig. 1). In order to meet the assumptions of ANOVA, the raw data were \log_e transformed. Effects of flower colour on the number of floral visitor species were also compared with ANOVA and with the Bonferroni test for multiple *a posteriori* comparisons among pairs of means. The statistical program package SPSS (10.0, 1999^[43]) was used.

Results

The three most common sugars (sucrose, glucose and fructose) were found in all except six species that had no sucrose (Table 1). In addition, six species showed an unidentified

monosaccharide in a low percentage (Table 1). There was a remarkable variation in the sugar proportions among the studied taxa (range of variation 2.3–56.5% for fructose; 3.2–67.3% for glucose and 0–90.3% for sucrose). In general, sugar proportions were constant when two or more samples per species were analysed. Only two species (*Vernonia mollissima* and *Bidens laevis*) showed large sugar variations among samples. In two other species (*Wedelia buphtalmiflora* and *Stevia satpureifolia*), one of the studied samples showed sucrose while in others it was absent (Table 1). The nectar of the majority of taxa (77%) present a larger proportion of hexoses than sucrose. Only eight species offer nectar with > 35% sucrose (*Carduus thoermeri*, *Vernonia fulva*, *V. nudiflora*, *Acmella decumbens* var. *affinis*, *Bidens laevis*, *Zinnia peruviana* and *Eupatorium viscidum*). Hexose ratios showed that in most species (83%) glucose predominates over fructose (Table 1).

Table 3 Correlation analyses between nectar sugar composition, corolla tube length and the number of floral visitor species in 35 Asteraceae species. Abbreviations: Sr = sucrose/(fructose+glucose), Hr = glucose/fructose, r = correlation coefficient, N = number of plant species, r_c = correlation coefficient corrected for phylogeny (in all cases $N = 5$, corresponding to five monophyletic groups of species, see Fig. 1), * $p < 0.05$

	Corolla tube length (mm)	Sucrose (%)	Fructose (%)	Glucose (%)	Sr	Hr
Number of visitor species	$r = -0.27$ $N = (29)$ $r_c = -0.26$	$r = -0.19$ $N = (25)$ $r_c = -0.30$	$r = 0.23$ $N = (25)$ $r_c = 0.20$	$r = 0.16$ $N = (25)$ $r_c = 0.33$	$r = -0.25$ $N = (25)$ $r_c = -0.18$	$r = -0.29$ $N = (25)$ $r_c = 0.18$
Corolla tube length (mm)		$r = 0.02$ $N = (31)$ $r_c = 0.89^*$	$r = -0.02$ $N = (31)$ $r_c = -0.74$	$r = -0.01$ $N = (31)$ $r_c = -0.90^*$	$r = 0.02$ $N = (31)$ $r_c = 0.25$	$r = 0.06$ $N = (31)$ $r_c = -0.68$

There was also a considerable variation in corolla tube length among the different taxa (range of variation 1.4–19.7 mm, Table 2). All species were visited by numerous insects belonging to ≥ 2 different orders (except for *Cichorium intybus*, which was visited only by hymenopterans). The 44% of total insect visits were made by hymenopterans, 24% by lepidopterans, 21% by dipterans, and 11% by coleopterans (Table 2).

At the species level, no significant correlations were found between the number of floral visitor species, nectar sugar proportions and corolla tube length (Table 3). Additional analyses were made in order to assess the effects of phylogenetic relatedness (only five monophyletic groups were considered; see "Materials and Methods") which showed significant correlations between corolla length and nectar sugar composition. The monophyletic groups with longer corollas tend to have higher nectar sucrose proportions (Table 3).

Nested ANOVA results showed that there were no significant differences among subgroups of species (monophyletic groups of tribes). However, corolla length showed significant differences between the two larger groups considered (Table 4). Early-branching species have longer corollas than late-branching ones (Fig. 2). Although statistical differences were not significant, it is also worth considering that early-branching species tend to have higher nectar sucrose proportions and lower floral visitor species than late-branching species (Fig. 2).

On the other hand, significant differences were found in the number of insect species that visit the flowers of different colours ($F_{2,26} = 4.80$, $p < 0.02$; colour categories: a. white, b. yellow and c. pink, lilac, purple or sky blue flowers). *A. posteriori* comparisons showed that white flowers were visited by a larger number of insect species than pink, lilac, purple or sky blue flowers.

Discussion

According to our data, Asteraceae species showed a large variability in the nectar sugar composition. Although most species presented hexose predominance, we cannot confirm that sugar ratios are phylogenetically conservative at the family level, as previously thought (Baker and Baker, 1983b^[4]). We found many taxa (23%) which showed nectars with large proportions of sucrose. In addition, we failed to find statistically significant differences in sugar ratios between early- and late-branching species. Finally, if we analyse nectar sugar composition at the tribe level, including data for 75 Asteraceae species previously

Table 4 Nested analysis of variance for nectar traits, corolla tube length and floral visitor species. Between group comparisons correspond to early- and late-branching species of Asteraceae (group 1 = tribe Mutisieae, subfam. Cardioideae, and subfam. Cichorioideae; group 2 = subfam. Asteroideae). Among subgroup comparisons correspond to five monophyletic groups of species (see Fig. 1). Values are F ratios with 1 df for between group comparisons and 3 df for among subgroup comparisons. Abbreviations: Sr = sucrose/(fructose + glucose), Hr = glucose/fructose, * $p < 0.05$

	Source of variation	
	Between groups	Among subgroups within groups
Sucrose (%)	2.839	0.442
Fructose (%)	2.084	0.739
Glucose (%)	3.115	0.651
Sr	3.926	0.619
Hr	0.049	0.904
Corolla tube length (mm)	12.215*	1.464
Number of visitor species	3.363	0.872

studied (Wykes, 1952^[52]; Percival, 1961^[33]; Van Handel et al., 1972^[50]; Hainsworth and Wolf, 1976^[21]; Kapila, 1978^[26]; Neff and Simpson, 1990^[32]; Pham-Delegue et al., 1990^[35]; Bernardello et al., 1994^[7], 1999^[8]; Galetto, 1995^[17]; Baker et al., 1998^[6]; Torres, 1998^[49]), only species of the tribes *Astereae*, *Anthemideae*, *Senecioneae* and *Helenieae* showed a clear dominance of monosaccharides. Species of the tribes *Cardueae* and *Inuleae* had, in general, similar quantities of mono- and disaccharides, while species of the tribes *Mutisieae*, *Lactuceae*, *Heliantheae* and *Eupatorieae* had nectars with a great variation in sucrose percentages.

In contrast, corolla tube length seems to be phylogenetically conservative in the studied taxa. The early-branching clades have longer corollas than late-branching ones. Data also showed a tendency for lower sucrose percentages and larger number of floral visitor species in late- than in early-branching clades (Fig. 2), although significant differences were not detected.

In summary, results showed that pollinators are not an important driving force behind variation in the composition of nectar and corolla tube length. However, floral traits seem to be linked to the phylogeny of the species. Late-branching species tend to have shorter corollas, lower sucrose proportions and

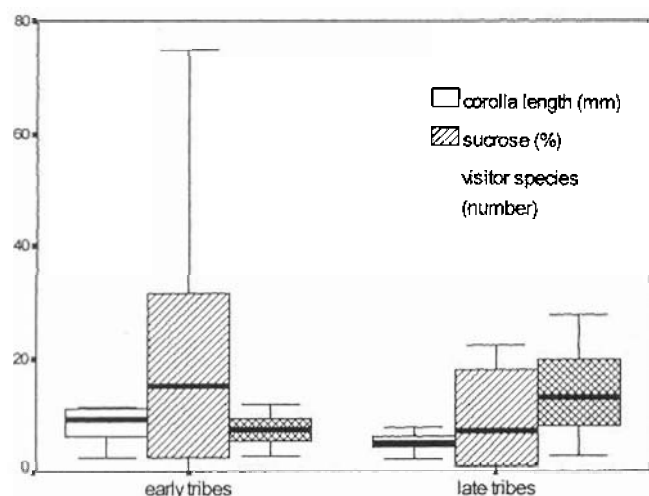


Fig. 2 Box plots for corolla tube length, nectar sucrose proportions and number of floral visitor species comparing early- and late-branching species of Asteraceae (see Fig. 1). Early tribes = Mutisieae, Cardueae, Lactuceae and Vernonieae; late tribes = Astereae, Senecioideae, Helenieae, Heliantheae and Eupatorieae.

greater diversity of floral visitors than early-branching species. Considering that longer corolla tubes and higher nectar sucrose percentages may indicate some specialization in the pollination system (e.g., Baker and Baker, 1990^[5]; Goulson, 1999^[20]), we suggest that there is an evolutionary tendency toward generalist pollination systems within the family.

For a long time, studies on flowers and their animal visitors have led to the assumption that necessarily there are coevolutionary relationships between floral traits and pollinator type (Percival, 1965^[34]; Faegri and van der Pijl, 1966^[12]; Proctor and Yeo, 1973^[37]). Therefore, many readers may feel that more complex flowers within a family and more evolutionarily derived families are necessarily more specialized, and so on. For example, on the basis of this theory but with few quantitative data, Mani and Saravanan (1999^[28]) interpreted that there is a very pronounced trend in Asteraceae heads to develop floral characters to favour more specialized butterfly pollination. In contrast, our results showed that members of many insect groups visit asteraceous heads, as has been previously noted (Lane, 1996^[27] and references therein). Our data also indicate that derived species are visited by a larger diversity of insects than basal ones, and some floral characters, such as corolla tube length and nectar sugar composition, tend to evolve to favour the visit of more insect species. Recent examples in other families also show reversals in the pollination system from extreme specialization to generalization (e.g., Armbruster and Baldwin, 1998^[2]).

There are several general and mutually non-exclusive hypotheses that can be considered to explain the suggested evolution of Asteraceae toward a generalist pollination system. According to Herrera (1996^[24]), plants may be quite successfully pollinated even though the floral traits at work did not actually evolve in relation to their present pollinators. In this way, it would be risky to conclude that lineages always evolve toward specialization (Waser et al., 1996^[51]). It must be also considered that ecological factors may constrain adaptive responses of plants to selection by pollinators, even when selection actu-

ally occurs (Herrera, 1996^[24]). Corolla and floral reward traits may have not only attractive functions but also fulfill a number of other ecological functions (Galen, 1999^[15]).

Finally, it is important to take into account that species commonly interact with many other species; therefore, plants can specialize and coevolve with multiple pollinator species within natural populations (Thompson, 1999^[48]). Perhaps asteraceous species are highly adapted to being pollinated by a wide variety of animal visitors. Perhaps it is through this mechanism rather than specialization of pollination systems that actual asteraceous diversification has originated.

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C. Torres

Instituto Multidisciplinario de Biología Vegetal
(Universidad Nacional de Córdoba-CONICET)
Casilla de Correo 495
5000, Córdoba
Argentina

E-mail: ctorres@imbiv.unc.edu.ar
leo@imbiv.unc.edu.ar

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