

# Phylogenetic analysis of interspecific variation in nectar of hummingbird-visited plants

J. F. ORNELAS,\* M. ORDANO,\* A. J. DE-NOVA,\* M. E. QUINTERO\* & T. GARLAND JR†

\*Departamento de Biología Evolutiva, Instituto de Ecología, A.C., Xalapa, Veracruz, México

†Department of Biology, University of California, Riverside, CA, USA

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sugar production.

## Abstract

We tested whether phylogeny, flower size and/or altitude were significant predictors of interspecific variation in nectar production of hummingbird-visited plants in an assembled database (289 species, in 22 orders, 56 families and 131 genera). Although the study is focused on hummingbird-pollinated plants (241 plant species), plants with different pollinator syndromes (48 species) are also included in the analyses. Nectar volume secreted in a given time period (usually 24 h) by a given flower, its sugar concentration and corolla length were compiled mainly from the literature. Altitude was also obtained from the original references. Sugar production was computed basically as the product of nectar secretion and sugar concentration, and expressed on a per 24-h basis. All nectar traits and corolla length (all log transformed), as well as altitude, showed statistically significant phylogenetic signal. Both nonphylogenetic and phylogenetically informed (independent contrasts) analyses indicated a highly significant positive correlation between corolla length and both nectar volume and sugar production. In addition, altitude (which is partially a surrogate for temperature) was significantly negatively correlated with both sugar concentration and production. Possible reasons for coadaptation of nectar production and sugar production with corolla length are discussed.

## Introduction

Nectar is the most ubiquitous floral reward among animal-pollinated angiosperms (Cruden *et al.*, 1983; Simpson & Neff, 1983), and its provision within flowers influences components of pollination visitation, including plant choice, foraging time and foraging movements (e.g. Pyke, 1984; Zimmerman, 1988; Mitchell, 1994). Increased visitation rates might prove beneficial to plants in two ways: (1) the probabilities of pollen import and

export may increase with visitation rates (Zimmerman, 1988; Mitchell, 1994); and (2) the plant can become selective as to which pollen grains fertilize the ovules, leading to higher seed quality (e.g. Mulcahy, 1979; Stanton *et al.*, 1986; Stephenson *et al.*, 1988; but see Klinkhamer *et al.*, 1994; Mitchell, 1994). However, greater allocation of resources to increasing nectar production involves an energy investment (Pleasants & Chaplin, 1983; Southwick, 1984), which can entail a reproductive cost (Pyke, 1991; Ordano & Ornelas, 2005).

Hummingbird-visited flowers, which produce relatively large amounts of easily collected nectar, are particularly appropriate for a detailed quantitative analysis of interspecific variation in nectar production rates (hereafter NPR). The quantity of nectar varies widely among species (Cruden *et al.*, 1983; Pleasants, 1983); among crops have commonly between 0.1 and 10  $\mu\text{L}$ , but nectar accumulation can reach > 650  $\mu\text{L}$  in flowers of some columnar cacti and agaves commonly visited by

*Correspondence:* Juan Francisco Ornelas, Departamento de Biología Evolutiva, Instituto de Ecología, AC, Km 2.5 Carretera Antigua a Coatepec No. 351, Congregación El Haya, Apartado Postal 63, Xalapa, Veracruz 91070, México.

Tel.: +11 52 228 842 1800 ext. 3000; fax: +11 52 228 818 7809;

e-mail: francisco.ornelas@inecol.edu.mx

Present address: M. E. Quintero, Department of Ornithology, American Museum of Natural History, Central Park West at 79 Street, New York, NY 10024, USA.

hummingbirds during daylight hours (e.g. Cruden *et al.*, 1983). Several authors have suggested that there is considerable opportunity for adaptive divergence of NPR through pollinator-mediated selection (e.g. Schemske & Bradshaw, 1999) and, accordingly, most workers have interpreted interspecific variation in NPR in terms of adaptations of plants to their pollinators (reviewed by Nicolson & Fleming, 2003; Pacini *et al.*, 2003). This may be especially true with respect to hummingbirds, because they are obligate nectar feeders that distinguish between rich and poor nectar phenotypes over space (Sutherland & Gass, 1995; Irwin & Brody, 1999; Schemske & Bradshaw, 1999) and have exceptionally high energy requirements (Brice, 1992; Altshuler *et al.*, 2004 and references therein).

The simplest selection scenario (involving rate of pollinator visitation) would suggest certain correlations between plant traits and between plant traits and environmental factors. Flowers that have presumably coevolved with pollination by hummingbirds provide both energy and water that could be balanced simultaneously by producing nectars of appropriate sugar concentrations, depending on the ambient temperature (Baker, 1975; Calder, 1979; Pyke & Waser, 1981). This might lead one to expect higher nectar concentration at higher elevation, but simultaneously balancing evaporative water loss with attainment of energy balance by a bird. Given that the rate of nectar uptake by hummingbirds is dependent on its viscosity (Kingsolver & Daniel, 1983), which increases with decreasing temperature (Calder, 1979), it is predicted that nectar concentration in hummingbird flowers will decrease with increasing altitude to compensate for the higher viscosity at lower temperatures (Calder, 1979). Because altitude is generally negatively correlated with temperature (higher is colder), this prediction can be indirectly tested by correlating nectar concentration with altitude. No such relationships between habitat (temperature or altitude) and nectar concentration have yet been found (Hainsworth, 1973; Calder, 1979; Pyke & Waser, 1981; but see Stiles & Freeman, 1993).

During the past three decades, evolutionary biologists have developed a large body of theory and empirical work for explaining the evolution of nectar rewards, particularly NPR, in terms of species differences in the selection of pollinators, in food preferences, or in response to such ecological conditions as competition for pollinators, their population densities and environmental factors (e.g. temperature and altitude: reviewed by Baker & Baker, 1983; Cruden *et al.*, 1983; Stiles & Freeman, 1993; Nicolson & Fleming, 2003; Pacini *et al.*, 2003; Lotz & Schondube, 2006). Following the development of evolutionary quantitative genetics in the 1980s (e.g. Roff, 1997), the focus of studies on the evolution of NPR shifted somewhat from traditional, ecological hypothesis with simple selection scenarios (e.g. involving rate of pollinator visitation) to other hypotheses and

experimental work with more elaborate and realistic scenarios to elucidate proximate factors underlying the evolution of nectar. In this period, a number of workers explored natural selection in the wild, including pollinator-mediated selection on floral traits (e.g. Harder & Cruzan, 1990; Mitchell & Shaw, 1993; Stanton & Young, 1994; Campbell, 1996; Galen & Cuba, 2001), and erected adaptive hypotheses for such traits as flower size, NPR and observed correlations among such traits. Here, we examine patterns in trait variation and covariation among plant species by use of phylogenetically based statistical methods. Our general objective was to test both adaptive and nonadaptive hypotheses related to nectar among hummingbird-visited plants from various geographic regions of the New World. We contrast the adaptationist hypothesis that the correlation of corolla length with nectar is mediated by selection on the part of larger bodied pollinators for longer flowers and higher reward against competing hypotheses based only on flower allometry, genetic correlations between these traits and evolutionary history. Specifically, a hummingbird's use of a given pool of flower species may not result from active choice but rather may simply be a consequence of the scaling of corolla length (and/or nectary size) and nectar such that longer flowers are always richer in nectar (i.e. allometric relationships) and/or constrained by genetic correlations. Alternatively, the correlation between corolla length with nectar could be explained by variation in resource availability of different habitats (i.e. altitude) in which shifts into richer habitat could have allowed production of larger flowers and more nectar, rather than mediated by hummingbirds or differences in the evolutionary history of plant species.

Our focus in this paper was on variation of NPRs (nectar volume and sugar per flower) and sugar concentration because these nectar traits are of direct significance to pollinator energetics (e.g. Heinrich & Raven, 1972; Heinrich, 1975; Feinsinger, 1978; Rathcke, 1992) and have been shown to influence pollinator visits (Schemske & Bradshaw, 1999; Bradshaw & Schemske, 2003). In addition, NPRs are the most variable of functionally related floral attributes (Cresswell, 1998) and have been shown to be heritable to some degree in some plant species (reviewed in Mitchell, 2004; see also Leiss *et al.*, 2004). Other things being equal (e.g. the number of flowers on offer), we predict a positive correlation between corolla length and both nectar production and sugar production rate as the result of selection by larger bodied pollinators for larger flowers and more energetically rewarding nectar. Correlated evolution of these flower characteristics might occur if hummingbird preference has indeed led to the evolution of higher NPR. This is the first attempt to distinguish among putative explanations for variation and covariation in nectar characteristics, considering flower size, environment (altitude) and phylogeny.

## Methods

### Data

We compiled a database for hummingbird-visited plants mainly from the literature (references listed in Appendix: Table A1), with some additions of our own work. A total of 289 species, in 22 orders, 56 families and 131 genera were included in the analysis (see Appendix: Table A2). Herbs (44.6%) dominated the species sample, with lesser representation of shrubs (18.7), trees (18.3), epiphytes (13.5) and lianas (6.3). Taxonomically, the sample was dominated by the orders Lamiales (77 species), Zingiberales (31), Poales (28), Fabales (26), Gentianales (17), Ericales (16), Asparagales (15) and Asterales (13). Most common families included Bromeliaceae (29), Gesneriaceae (27), Fabaceae (26), Heliconiaceae (24), Acanthaceae (15), Scrophulariaceae (14) and Rubiaceae (13). Most of the data come from geographic areas in the USA, Mexico, Costa Rica and Brazil.

Information for most species includes altitude (metres above sea level), nectar production ( $\mu\text{L day}^{-1}$  per flower), sugar concentration (% w/w), sugar production ( $\text{mg day}^{-1}$  per flower) and corolla length (mm). We used nectar production and sugar production as the currency of pollinator energetics. Nectar production was calculated from the literature as the volume of nectar secreted in a given time period. We accounted for variation in how long nectar was measured by correcting all values to be per 24 hours. Total sugar production (mg sugar) was the product of nectar volume (mL) and concentration per unit volume (mg per mL). Values were first converted to mg sugar per 100 mL nectar with the table in the CRC Handbook of Chemistry and Physics (1978) according to Bolten *et al.* (1979), then multiplied by total nectar volume (see also Kearns & Inouye, 1993). Information on other traits influencing variation in nectar secretion patterns such as flower longevity, flower age, flower's sexual phase, floral morph, response to nectar removal and temporal patterns were rarely mentioned in the references. Nectar measurements were generally done on different flowers in which nectar accumulates over time; however, researchers sampled repeatedly the same flowers over time in some of the studies (< 10%). These two nectar-sampling methodologies might have introduced some bias in our study because some hummingbird-visited plants respond positively by replenishing nectar after repeated removal (Ordano & Ornelas, 2004 and references therein). Many plant species in our database have flowers that attract a broad spectrum of visitors, and hummingbirds sometimes visit flowers with a suite of characteristics that do not correspond to the typical floral syndrome of hummingbird pollination (Faegri & van der Pijl, 1979); overlap between floral syndromes exists in our data set. The entire data set is listed in Appendix (Table A2). It includes data generated during this study and data obtained from the literature.

Species in our sample were categorized into five broad pollination groups: (1) hermit type, if mainly visited by hermit hummingbirds (Subfamily Phaethorninae, 82 species); (2) trochiline type, if mainly visited by trochiline hummingbirds (Subfamily Trochilinae, 156 species); (3) passerine type, if mainly visited by perching birds (10 species); (4) bat type, if mainly visited by bats (10 species); and (5) insect type of flower, if mainly visited by insects (23 species). Plant species were categorized by its habitat as xeric (including páramo and tropical dry forest), mesic (e.g. pine-oak forest) or hydric (tropical rain forest, cloud forest) and its elevation in 500-m intervals from sea level to above 3000 m above sea level.

### Statistical analysis

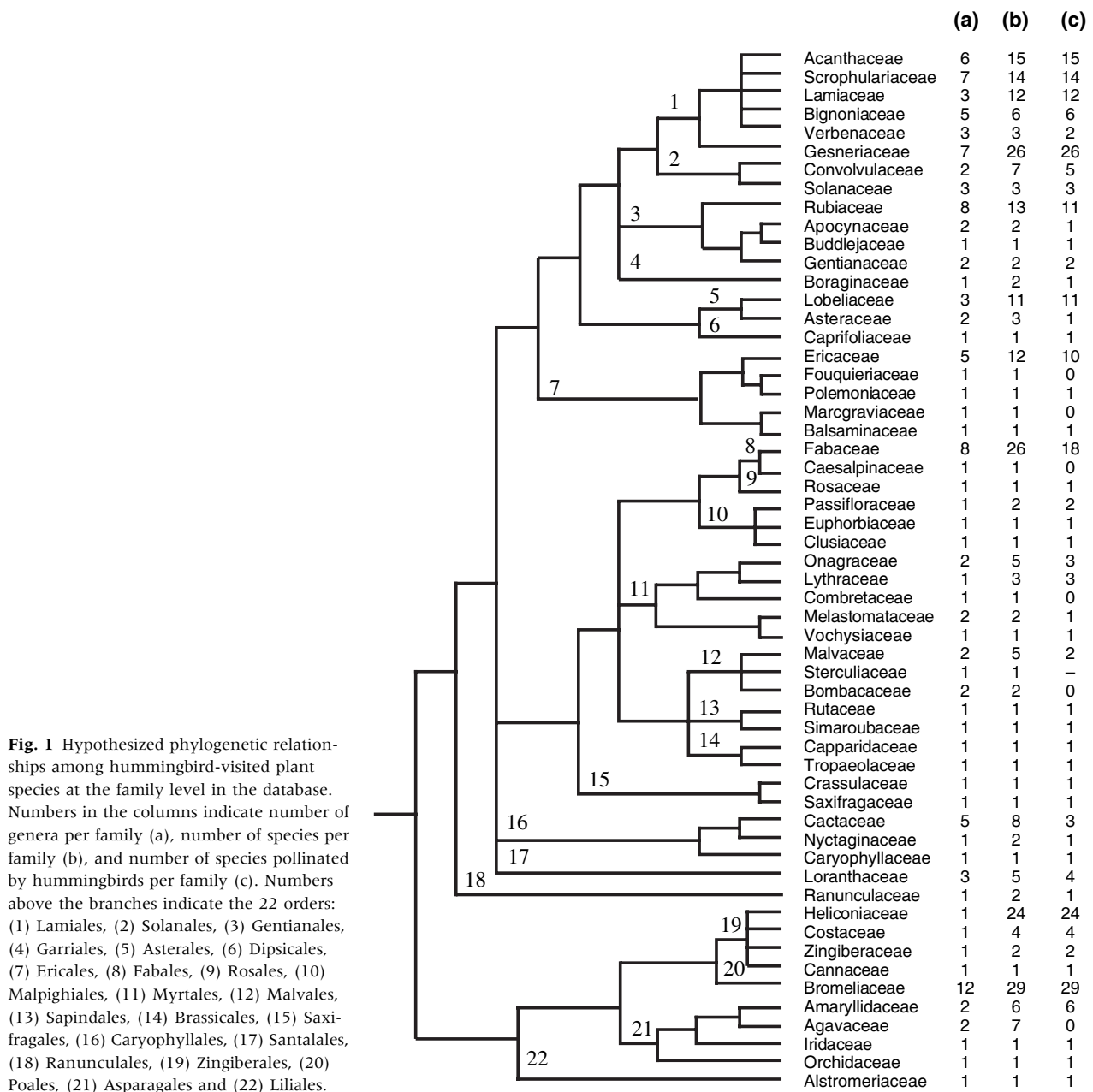
Because closely related species tend to share similar trait values (i.e. exhibit 'phylogenetic signal' *sensu* Blomberg *et al.*, 2003), comparative data sets may violate the assumptions of conventional statistical methods (Felsenstein, 1985; Grafen, 1989; Martins & Garland, 1991; Purvis *et al.*, 1994; Garland *et al.*, 2005). We therefore analysed pairwise relations using both conventional nonphylogenetic Pearson's correlations and the equivalent with phylogenetically independent contrasts (PICs) (Felsenstein, 1985; Garland *et al.*, 1992). Independent contrasts were calculated by use of the PDTREE program (Garland *et al.*, 1999), as a module of Mesquite (Maddison & Maddison, 2004). Conventional statistical analyses were done using StatView and SuperAnova (Abacus Concepts, Inc., Berkeley, CA, USA), as well as PDTREE. In its simplest form, Felsenstein's (1985) method assumes that the evolution of continuous characters can be modelled as a random walk process in continuous time, i.e. Brownian motion. This provides a strong reason for logarithmic transformation of size data in comparative analyses (Purvis & Rambaut, 1995). Therefore, nectar volume, sugar production, sugar concentration and corolla length data were  $\log_{10}$  transformed before all analyses. Because the phylogenetic tree used for analyses (see next paragraph) included many soft polytomies (indicating uncertainty about phylogenetic relationships), we bounded the degrees of freedom (d.f.) used for hypothesis testing as recommended by Purvis & Garland (1993) (see also Garland & Díaz-Uriarte, 1999).

We constructed a composite phylogeny that included all species represented in our data set based on the angiosperm higher-level topologies developed by Chase *et al.* (1993), Soltis *et al.* (2000) and Davies *et al.* (2004). We used these phylogenetic hypotheses to represent the backbone of the tree (deeper nodes) because their phylogenies achieve the most complete taxon sampling at higher taxonomic levels. For shallower taxonomic levels, we derived intrafamilial and intrageneric relationships from more narrowly focused phylogenetic studies. We used the online software Phylomatic (<http://www.phylodiversity.net/phylomatic/phylomatic.html>)

developed by Webb & Donoghue (2002) to rapidly assemble our phylogenetic tree from their mega-tree assembled 'by hand' in April 2004, rather than by an automated supertree algorithm (Sanderson *et al.*, 1998). The tree created by Phylomatic used information from numerous published molecular phylogenies to create a tree containing all of the species in the data set. In the absence of detailed information on phylogenetic relationships within many of the 56 families and 131 genera found in our data set, we assumed most families and genera were monophyletic and polytomous when placed

them on the tree. The resulting phylogenetic tree (Fig. 1; also see Appendix: Table A3) was used for all subsequent analyses of correlated evolution. Figure 1 illustrates the phylogeny used at the family level indicating the number of genera and number of species per family included in the study and whether they contain species pollinated by hummingbirds.

Because the phylogenetic information came from multiple studies using different methods, consistent estimates of branch length were not available. Therefore, four different types of arbitrary branch lengths were



considered: all = 1 (constant), Grafen (1989), Pagel (1992) and Nee (cited in Purvis *et al.*, 1994, p. 299). Based on the diagnostic of Garland *et al.* (1992), which checks for patterns in the plots of absolute values of standardized contrasts vs. their standard deviations, constant branch lengths worked the best and were used in all analyses. Constant branch lengths correspond to a 'punctuational' mode of character evolution (Harvey & Pagel, 1991; Martins & Garland, 1991). The 'punctuational' model is simply a Brownian motion model on a phylogeny with all branch lengths set equal to unity in which changes in character states occur only associated with speciation events, i.e. change could occur in both daughter species, and is better referred to as a 'speciational' model (for further discussion see Díaz-Uriarte & Garland, 1996).

We used a randomization procedure to test whether each trait studied exhibited a significant tendency for related species to resemble each other (phylogenetic signal). As described in Blomberg *et al.* (2003) (MatLab program PHYSIG.M), this test asks whether a given phylogeny (topology and branch lengths) better fits a set of tip data when compared with the fit obtained when the data have been randomly permuted across the tips of the tree, thus destroying any phylogenetic signal that may have existed (we used 1000 randomized data sets for these tests).

Because NPR evolution is perhaps best viewed as the product of evolutionary change in multiple potentially independent traits with multiple constraints, we also conducted multiple regression analyses. However, in this case we had to 'prune' the tree down to include only those species for which all species have data for all variables in the analysis.

## Results

Group means ( $\pm$ SD) of nectar production, sugar concentration, sugar production and corolla length of all species in the data set sorted by: (a) pollinator type; (b) altitude; (c) habitat; (d) habit; and (e) flower colour are presented in Table 1. The data are clearly distorted by the inclusion of certain values. For example, high NPR values in xeric habitats are influenced by the inclusion of data from *Erythrina oliviae*, *Pseudobombax ellipticum*, agaves (*Agave* and *Manfreda*) and columnar cacti (*Neobuxbaumia*), whereas high NPR values in trees are influenced by the 15 *Erythrina* trees, *P. ellipticum*, *Ceiba pentandra*, *Cordia rickseckeri* and the inclusion of agaves and columnar cacti (13 species) in that category (see also Table A2).

Nectar production rate values ranged more widely within plant groups (Tables 2 and 3), yielding an average standard deviation of 82  $\mu$ L for the 32 families in which multiple genera were sampled and 73  $\mu$ L for the 19 orders in which multiple families were sampled. Among orders, the Asparagales (Agavaceae) and Caryophyllales (Cactaceae) were nectar 'superproducers',

whereas Myrtales (e.g. Lythraceae and Onagraceae) and Lamiales (Acanthaceae, Bignoniaceae, Gesneriaceae, Lamiaceae and Scrophulariaceae) were relatively poor nectar producers (Table 2). The high NPR value for Malvales is due to the inclusion of two tree species in the Bombacaceae (*C. pentandra* and *P. ellipticum*). Relatively high NPR values (volume and sugar) are also observed among the Zingiberales (Heliconiaceae and Costaceae) and Fabales (mainly *Calliandra* and *Erythrina* species; see also Table A2). High corolla length values were observed for Amaryllidaceae (*Bomarea* and *Hippeastrum*), Bignoniaceae and Loranthaceae (*Gaiadendron* and *Psittacanthus*) with moderate amounts of nectar (Table 3; see also Table A2).

## Phylogenetic signal

As shown in Table 4, all traits showed statistically phylogenetic signal (all  $P < 0.001$ ), with the descriptive  $K$ -statistic ranging in the region of 0.3–0.4. For all traits, the hierarchical phylogenetic tree gave a better fit to the tip data than did a star phylogeny, as indicated by lower MSE (Table 4). Therefore, the results of phylogenetically based statistical analyses should generally be more reliable. Note that transformations of the branch lengths would have yielded even better fits of the hierarchical trees (Blomberg *et al.*, 2003), but we did not implement such transformations in the present study.

## Trait correlations

Based on conventional statistical analyses, all pairwise trait correlations were statistically significant, with the exception of sugar concentration and nectar production (Table 5). PICs tended to indicate somewhat weaker relations among traits, and in several cases nonsignificant ones if the minimum d.f. are considered. Considering the phylogenetic results (Table 5), altitude showed a weak but statistically significant negative relation with sugar concentration and also sugar production. Nectar production and corolla length were positively related, indicating that, as might be expected, larger flowers tend to produce more nectar. We found no significant linear correlation between nectar production and sugar concentration in our data. Corolla length was not correlated with sugar concentration, but was correlated with both nectar production and sugar production.

Using species values, we found that a full regression model that included sugar concentration, corolla length and altitude (all log transformed) explained a small amount of the variation in nectar production (adjusted  $r^2 = 0.19$ ). The  $P$  values in the regression coefficients showed that corolla length is the only statistically significant ( $P < 0.05$ ) variable predicting nectar volume (Table 6). In the case of sugar concentration, the adjusted  $r^2$  (0.05) indicates a very weak overall relationship. The  $P$  values in the regression coefficients showed that corolla

**Table 1** Nectar and flower length data of all species in the data set sorted by (a) pollinator type, (b) altitude, (c) habitat, (d) habit and (e) flower colour.

	Nectar production ( $\mu\text{L day}^{-1}$ per flower)		Sugar concentration (% w/w)		Sugar production (mg day <sup>-1</sup> per flower)		Corolla length (mm)	
	<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD
All data	278	41.6 $\pm$ 85.6	272	23.8 $\pm$ 7.8	263	9.4 $\pm$ 16.6	197	32.6 $\pm$ 17.2
Pollinator type								
Trochilines	156	22.5 $\pm$ 33.9a	156	23.5 $\pm$ 6.9a	153	5.9 $\pm$ 8.5a	109	34.8 $\pm$ 37.2a
Hermits	82	37.2 $\pm$ 25.0a	78	25.7 $\pm$ 6.4a	78	10.5 $\pm$ 8.4a	62	40.6 $\pm$ 12.8b
Perching birds	10	196.9 $\pm$ 217.8b	9	13.2 $\pm$ 2.8b	9	30.5 $\pm$ 35.9b	4	34.8 $\pm$ 37.2ab
Bats	10	265.3 $\pm$ 247.2b	9	16.1 $\pm$ 3.9b	9	44.7 $\pm$ 58.9b	7	39.3 $\pm$ 15.7ab
Insects	23	19.5 $\pm$ 26.9a	20	26.1 $\pm$ 13.4a	19	4.9 $\pm$ 6.7a	16	24.7 $\pm$ 14.5a
Altitude								
Low	101	37.9 $\pm$ 41.4a	100	26.2 $\pm$ 6.3a	100	10.8 $\pm$ 10.7a	80	34.1 $\pm$ 16.4a
Medium	83	52.2 $\pm$ 112.2a	76	22.3 $\pm$ 8.6b	76	10.3 $\pm$ 24.3ab	63	36.2 $\pm$ 19.4a
High	48	43.4 $\pm$ 97.6a	49	22.4 $\pm$ 6.7b	48	8.7 $\pm$ 14.8ab	27	25.1 $\pm$ 13.1b
Very high	44	26.3 $\pm$ 90.7a	44	22.4 $\pm$ 8.8b	42	4.6 $\pm$ 11.7b	27	24.7 $\pm$ 13.9b
Habitat								
Xeric	62	76.9 $\pm$ 135.9a	56	22.9 $\pm$ 1.3a	54	14.2 $\pm$ 31.8a	50	32.6 $\pm$ 19.6a
Mesic	138	26.9 $\pm$ 31.9b	137	22.9 $\pm$ 0.7a	135	6.6 $\pm$ 7.6b	93	30.1 $\pm$ 17.4a
Wet	79	35.5 $\pm$ 31.5b	76	25.6 $\pm$ 0.6b	76	10.0 $\pm$ 9.3b	56	34.4 $\pm$ 12.7a
Habit								
Herb	116	29.3 $\pm$ 28.3a	110	25.1 $\pm$ 7.1a	109	8.2 $\pm$ 8.9a	82	34.3 $\pm$ 16.3a
Shrub	49	35.8 $\pm$ 62.2a	47	21.9 $\pm$ 9.4ab	46	8.1 $\pm$ 13.1a	32	22.3 $\pm$ 16.8b
Tree	46	108.6 $\pm$ 181.0b	43	20.5 $\pm$ 7.2b	42	19.3 $\pm$ 34.5b	30	38.6 $\pm$ 23.0a
Epiphyte	34	22.1 $\pm$ 19.8a	34	27.2 $\pm$ 6.1a	34	7.2 $\pm$ 7.1a	32	33.3 $\pm$ 11.8a
Vine	17	19.7 $\pm$ 15.9a	17	21.1 $\pm$ 13.1a	17	4.1 $\pm$ 3.2a	14	33.9 $\pm$ 14.3a
Flower colour								
Red	97	36.1 $\pm$ 71.1ab	97	24.0 $\pm$ 7.9a	96	8.4 $\pm$ 12.2a	79	32.1 $\pm$ 17.0a
Orange	19	28.5 $\pm$ 51.6ab	18	22.1 $\pm$ 13.1a	18	5.8 $\pm$ 7.2a	16	40.9 $\pm$ 26.1a
Pink	27	21.5 $\pm$ 15.0b	27	24.5 $\pm$ 6.3a	26	5.4 $\pm$ 3.8a	23	35.0 $\pm$ 16.4a
White	17	103.4 $\pm$ 191.3a	14	25.6 $\pm$ 8.8a	14	25.1 $\pm$ 47.9b	15	27.6 $\pm$ 19.8a
Yellow	48	75.6 $\pm$ 126ab	45	22.2 $\pm$ 6.2a	44	16.1 $\pm$ 20.8b	42	31.4 $\pm$ 14.6a
Other	12	14.9 $\pm$ 17.2b	12	26.8 $\pm$ 4.9a	12	4.4 $\pm$ 4.7a	11	25.2 $\pm$ 12.7a

Species in our sample were categorized into five broad pollination groups: (1) hermit type, if mainly visited by hermit hummingbirds (Subfamily Phaethorninae, 82 species); (2) trochiline type, if mainly visited by trochiline hummingbirds (Subfamily Trochilinae, 156 species); (3) passerine type, if mainly visited by perching birds (10 species); (4) bat type, if mainly visited by bats (10 species); and (5) insect type of flower, if mainly visited by insects (23 species). Plant species were categorized by its habitat as xeric (including páramo and tropical dry forest), mesic (e.g. pine-oak forest) or hydric (tropical rain forest and cloud forest), and its elevation in 500-m intervals from sea level to above 3000 m above sea level. Letters 'a' and 'b' denote differences among groups based on Tukey's HSD methods.

length and altitude are useful in predicting sugar concentration (Table 6). For sugar production, we obtained a moderate overall relationship ( $r^2 = 0.22$ ). The *P* values of the regression coefficients showed that corolla length was the most useful variable in predicting sugar production and altitude, although significant, had a smaller contribution in predicting sugar production (Table 6).

When using PICs, we found that both nectar production and sugar production were strongly correlated with corolla length, and altitude had no significant effect in explaining NPR (nectar and sugar production) (Table 6). Moreover, contrasts of sugar concentration were not significantly correlated with nectar production, corolla length or altitude (Table 6). As data showed phylogenetic signal, results from PICs are emphasized.

## Discussion

Both nonphylogenetic and phylogenetic analyses of the relation between corolla length and (1) nectar production and (2) sugar production for hummingbird-visited plant species indicated statistically significant positive relations. That the relations retained statistical significance after accounting for phylogeny suggests that these traits have undergone true correlated evolution. Possible mechanisms (not mutually exclusive) are positive genetic correlations or correlational selection. In general, barring a change in shape (becoming more bulbous), flowers with longer corollas will be able to hold more nectar or will have nectary tissue of larger size, and this simple physical relation should be reflected, to some extent, in a

**Table 2** Nectar and flower length data of all species in the data set sorted by order.

Order	Nectar production ( $\mu\text{L day}^{-1}$ per flower)		Sugar concentration (% w/w)		Sugar production (mg day <sup>-1</sup> per flower)		Corolla length (mm)	
	<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD
Asparagales	15	156.2 $\pm$ 195.1	15	15.3 $\pm$ 3.1	15	24.5 $\pm$ 30.4	7	34.0 $\pm$ 23.1
Asterales	13	22.1 $\pm$ 13.4	13	20.3 $\pm$ 6.0	13	5.2 $\pm$ 5.2	10	36.0 $\pm$ 10.4
Caryophyllales	9	146.0 $\pm$ 258.4	8	29.1 $\pm$ 7.4	7	32.2 $\pm$ 68.7	10	43.1 $\pm$ 17.0
Ericales	16	25.2 $\pm$ 35.7	16	24.5 $\pm$ 14.1	16	5.0 $\pm$ 4.9	8	20.2 $\pm$ 11.5
Fabales	26	53.1 $\pm$ 61.9	24	22.1 $\pm$ 7.2	23	10.4 $\pm$ 9.1	15	47.9 $\pm$ 25.9
Gentianales	13	26.4 $\pm$ 60.0	13	20.0 $\pm$ 7.8	13	7.2 $\pm$ 16.1	11	26.2 $\pm$ 20.5
Lamiales	76	15.4 $\pm$ 14.2	75	23.9 $\pm$ 5.7	75	4.1 $\pm$ 3.7	42	29.7 $\pm$ 13.0
Malvales	8	112.0 $\pm$ 200.1	7	18.5 $\pm$ 3.9	7	20.2 $\pm$ 28.3	5	27.8 $\pm$ 7.3
Myrtales	11	10.4 $\pm$ 10.2	9	21.7 $\pm$ 9.5	9	2.4 $\pm$ 1.6	9	21.2 $\pm$ 16.8
Poales	28	23.9 $\pm$ 19.5	28	27.7 $\pm$ 5.7	28	7.6 $\pm$ 6.5	26	33.6 $\pm$ 11.9
Santalales	5	14.1 $\pm$ 12.6	5	19.2 $\pm$ 1.3	4	3.4 $\pm$ 2.8	3	50.5 $\pm$ 39.4
Solanales	10	17.9 $\pm$ 17.2	10	26.9 $\pm$ 7.6	10	6.2 $\pm$ 6.4	8	29.0 $\pm$ 12.7
Zingiberales	31	63.1 $\pm$ 26.3	27	27.2 $\pm$ 4.3	27	19.7 $\pm$ 10.2	27	40.2 $\pm$ 12.2

Only orders with more than five species in the data set are shown.

**Table 3** Nectar and flower length data of all species in the data set sorted by family.

Order	Family	Nectar production ( $\mu\text{L day}^{-1}$ per flower)		Sugar concentration (% w/w)		Sugar production (mg day <sup>-1</sup> per flower)		Corolla length (mm)	
		<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD
Asparagales	Agavaceae	7	293.8 $\pm$ 214.9	7	14.5 $\pm$ 2.1	7	45.2 $\pm$ 34.2	2	22.5 $\pm$ 10.6
	Amaryllidaceae	6	37.7 $\pm$ 32.8	6	15.2 $\pm$ 2.8	6	5.9 $\pm$ 5.0	4	45.1 $\pm$ 25.1
Asterales	Lobeliaceae	11	25.3 $\pm$ 11.9	10	19.9 $\pm$ 6.7	11	5.9 $\pm$ 5.4	8	32.9 $\pm$ 9.2
Caryophyllales	Cactaceae	6	216.9 $\pm$ 297.9	5	31.4 $\pm$ 8.3	4	55.3 $\pm$ 88.2	7	50.9 $\pm$ 11.9
Ericales	Ericaceae	12	19.7 $\pm$ 13.7	12	20.1 $\pm$ 3.6	12	4.4 $\pm$ 3.2	4	20.9 $\pm$ 16.5
Fabales	Fabaceae	25	54.1 $\pm$ 62.9	24	22.1 $\pm$ 7.2	23	10.4 $\pm$ 9.1	14	48.7 $\pm$ 26.8
Gentianales	Rubiaceae	13	26.4 $\pm$ 60.0	13	20.0 $\pm$ 7.8	13	7.2 $\pm$ 16.1	11	26.2 $\pm$ 20.5
Lamiales	Acanthaceae	15	20.8 $\pm$ 19.6	15	21.5 $\pm$ 3.3	15	4.8 $\pm$ 4.4	11	31.9 $\pm$ 10.7
	Bignoniaceae	6	17.1 $\pm$ 9.8	6	27.3 $\pm$ 4.5	6	5.1 $\pm$ 2.6	6	41.5 $\pm$ 15.8
	Gesneriaceae	26	17.1 $\pm$ 11.8	26	25.7 $\pm$ 6.3	26	5.1 $\pm$ 3.7	9	30.8 $\pm$ 11.9
	Lamiaceae	12	10.0 $\pm$ 7.3	12	24.1 $\pm$ 4.2	12	2.5 $\pm$ 1.7	6	22.6 $\pm$ 7.4
	Scrophulariaceae	14	13.0 $\pm$ 17.0	13	22.2 $\pm$ 7.3	13	3.1 $\pm$ 3.9	9	24.8 $\pm$ 12.8
Malvales	Malvaceae	5	34.3 $\pm$ 24.8	5	19.3 $\pm$ 3.8	5	7.3 $\pm$ 6.0	4	28.2 $\pm$ 8.3
Myrtales	Lythraceae	3	7.8 $\pm$ 6.4	2	34.5 $\pm$ 12.4	2	3.9 $\pm$ 0.2	1	22.9 $\pm$ 0.0
	Onagraceae	4	14.8 $\pm$ 14.4	4	18.2 $\pm$ 6.1	4	2.4 $\pm$ 1.9	4	28.0 $\pm$ 23.6
Poales	Bromeliaceae	28	23.9 $\pm$ 19.5	28	27.7 $\pm$ 5.7	28	7.6 $\pm$ 6.5	26	33.6 $\pm$ 11.9
Santalales	Loranthaceae	5	14.1 $\pm$ 12.6	5	19.2 $\pm$ 1.2	4	3.4 $\pm$ 2.8	3	50.4 $\pm$ 39.4
Solanales	Convolvulaceae	7	21.9 $\pm$ 18.9	7	30.4 $\pm$ 5.1	7	7.9 $\pm$ 6.9	6	35.5 $\pm$ 3.6
Zingiberales	Costaceae	4	61.2 $\pm$ 22.9	4	30.0 $\pm$ 3.4	4	21.3 $\pm$ 9.6	3	47.9 $\pm$ 12.5
	Heliconiaceae	24	65.1 $\pm$ 25.9	20	27.0 $\pm$ 4.6	20	20.3 $\pm$ 10.4	23	40.2 $\pm$ 11.3

Only families with more than three species in the data set are shown.

positive genetic correlation. We are not aware of any study showing the genetic correlation between corolla length and NPR. Under controlled conditions, a genetic correlation between corolla length and NPR in hummingbird-pollinated *Penstemon centranthifolius* (Scrophulariaceae) was positive but not significantly different from zero (see Table 3 in Mitchell & Shaw, 1993 and Table 1 in Mitchell *et al.*, 1998). In bumblebee-pollinated *Echium vulgare* (Boraginaceae), no significant correlation

between NPR and measures of floral dimensions was found by Klinkhamer & van der Veen-van Wijk (1999) (see also Leiss *et al.*, 2004). Under natural conditions, no evidences for a genetic correlation between nectar volume and corolla length in hummingbird-pollinated *Ipomopsis aggregata* (Polemoniaceae) was found; however, their phenotypic correlation was moderately strong and positive, suggesting the possibility of common environmental effects on both of these traits (Campbell, 1996).

**Table 4** Diagnostic test for adequacy of branch lengths (all set equal to unity) with phylogenetically independent contrasts (Garland *et al.*, 1992), mean squared error of tree fitted to tip data (smaller indicates better fit), *K* statistic indicating amount of phylogenetic signal, and significance test for presence of phylogenetic signal (Blomberg *et al.*, 2003).

PDI file	<i>n</i>	Internal branches of zero length	Trait	Phylogeny	Diagnostic correlation*	MSE	<i>K</i>	<i>P</i> signal from randomization test
NEC283 L1.PDI	283	175	log Nectar Production ( $\mu\text{L day}^{-1}$ per flower)	Star Tree	0.037	0.310 0.244	0.433	< 0.001
CON266 L1.PDI	266	165	log Sugar Concentration (% w/w)	Star Tree	-0.013	0.0201 0.0181	0.284	< 0.001
SUG263 L1.PDI	263	163	log Sugar Production ( $\text{mg day}^{-1}$ per flower)	Star Tree	0.004	0.314 0.268	0.367	< 0.001
COR201 L1.PDI	201	113	log Corolla Length (mm)	Star Tree	-0.084	296.3 270.0	0.296	< 0.001
ALT272_1.PDI	272	165	Altitude (metres above sea level)	Star Tree	-0.124	$8.80 \times 10^5$ $7.94 \times 10^5$	0.345	< 0.001

\*Based on this diagnostic, none shows significant lack of fit to the tip data (all  $P > 0.05$ ).

**Table 5** Pairwise correlations of nectar production, sugar production, corolla length, sugar concentration and altitude based on nonphylogenetic and phylogenetic (independent contrasts) analyses.

	<i>n</i>	Conventional <i>r</i>	Conventional <i>P</i>	PIC, <i>r</i>	No. of zero-length branches	d.f. min	<i>P</i> , d.f. min	d.f. max	<i>P</i> , d.f. max
log VOL vs. log COR	193	0.472	$\ll 0.001$	0.391	109	82	< 0.001	191	< 0.001
log VOL vs. ALT	263	-0.228	< 0.001	-0.118	161	100	> 0.2	261	0.08
log VOL vs. log SUG	263	0.960	$\ll 0.001$	0.958	163	98	$\ll 0.001$	261	$\ll 0.001$
log SUG vs. log COR	180	0.481	$\ll 0.001$	0.390	100	78	< 0.001	178	< 0.001
log SUG vs. ALT	253	-0.289	$\ll 0.001$	-0.175	155	96	0.09	251	0.006
log COR vs. ALT	188	-0.259	< 0.001	-0.104	103	83	0.35	186	0.15
log CON vs. log VOL	262	-0.063	n.s.	-0.025	163	97	n.s.	260	n.s.
log CON vs. log COR	182	0.184	< 0.02	0.050	101	79	n.s.	180	n.s.
log CON vs. log SUG	263	0.211	< 0.001	0.254	163	98	0.01	261	< 0.001
log CON vs. ALT	256	-0.252	< 0.001	-0.202	157	97	< 0.05	254	0.001

VOL = nectar production ( $\mu\text{L day}^{-1}$  per flower), SUG = sugar production ( $\text{mg day}^{-1}$  per flower), COR = corolla length (mm), CON = sugar concentration (% w/w) and ALT = altitude (metres above sea level). Nectar, sugar, corolla length and concentration data were  $\log_{10}$  transformed. All branch lengths = 1 for phylogenetically independent contrasts (PIC). For PIC, d.f. min are ( $n - 2$  - number of zero-length branches) and d.f. max are ( $n - 2$ ) (see text; Purvis & Garland, 1993). All *P* values are for two-tailed tests.

**Table 6** Nonphylogenetic and phylogenetic multiple regressions with PICs of nectar traits (nectar production, sugar concentration and sugar production) with corolla length and altitude.

Nonphylogenetic (using species values)					Phylogenetic (using independent contrasts)				
Dependent	Independent	$\beta$	<i>F</i>	<i>P</i>	$\beta$	<i>F</i>	<i>P</i>	95% Confidence Intervals for $\beta$	
								Lower bound	Upper bound
Nectar production	CON	-0.09	1.93	0.1666	-0.08	1.37	0.243	-0.867	0.222
	COR	0.43	37.21	< 0.0001	0.33	20.47	0.0001	0.421	1.072
	ALT	-0.14	3.84	0.0519	-0.06	0.68	0.411	0.000	0.000
Sugar concentration	VOL	-0.11	1.93	0.1666	-0.09	1.37	0.243	-0.066	0.017
	COR	0.20	6.20	0.0137	0.057	0.50	0.479	-0.061	0.129
	ALT	-0.18	5.48	0.0207	0.128	2.86	0.093	0.000	0.000
Sugar production	COR	0.41	6.71	< 0.0001	0.33	20.71	0.0001	0.434	1.098
	ALT	-0.18	36.72	0.0103	-0.08	1.36	0.245	0.000	0.000

VOL = nectar production ( $\mu\text{L day}^{-1}$  per flower), SUG = sugar production ( $\text{mg day}^{-1}$  per flower), COR = corolla length (mm), CON = sugar concentration (% w/w), and ALT = altitude (metres above sea level). Nectar, sugar, corolla length and concentration data were  $\log_{10}$  transformed. All branch lengths = 1 and linear regressions through the origin for phylogenetically independent contrasts (PIC). Also note that no d.f. have been subtracted for polytomies. Regression coefficients are standardized.  $n = 174$  in all cases. All *P* values are for two-tailed tests.

That no one has shown a genetic correlation may simply reflect how few studies have looked for one. The idea that the correlation of corolla length with nectar could be produced by selection on the part of larger-bodied pollinators for larger flowers and higher reward is supported by phenotypic correlations. Indeed, phenotypic correlations between aspects of flower size (inflorance size, flower depth, flower width, petal size, corolla length and perianth area) and nectar traits have been shown for some plant species (Harder & Cruzan, 1990; Cresswell & Galen, 1991; Mitchell & Shaw, 1993; Stanton & Young, 1994; Campbell, 1996; Mitchell *et al.*, 1998; Worley & Barrett, 2000; but see Leiss *et al.*, 2004). Genetic correlations may either constrain or facilitate response to selection (Garland, 1994; Mitchell, 2004). Correlational selection (*sensu* Brodie *et al.*, 1995) may be acting through a relationship between tube length, tongue and/or bill lengths, pollinator size and energetic demands (Heinrich & Raven, 1972). In the following sections, we contrast adaptive and nonadaptive hypotheses related to variation in NPR.

In the present study, we have emphasized results of phylogenetically informed statistical analyses. This is generally justified because all of the traits studied showed highly significant phylogenetic signal, based on the randomization test of Blomberg *et al.* (2003) (see Table 4). Although highly statistically significant, the amount of phylogenetic signal in the traits studied, as indicated by *K* statistics ranging between 0.30 and 0.43, was substantially less than if the phylogenetic trees (and assumed Brownian motion model of character evolution) provided a 'perfect' fit to the tip data, in which case *K* = 1.00. However, the present *K* values are similar to those that have been reported previously in a broad survey of traits (see Fig. 6 in Blomberg *et al.*, 2003; also Rezende *et al.*, 2004). Phylogenetic signal lower than unity can result from several things, including adaptive evolution in response to natural selection, evolution in response to sexual selection, and various types of 'measurement error' (Blomberg *et al.*, 2003; Ives *et al.*, 2007). Measurement error is likely to be fairly large for our trait data because they were collected mainly from plants growing in nature rather than under common-garden conditions (Garland & Adolph, 1994; Mitchell, 2004), by a variety of workers, using somewhat varied techniques. It is possible that the type of measurement employed in our study (accumulated nectar) poorly represents the real variation in nectar production dynamics because of among-species variation in post-secretory processes (e.g. reabsorption, evaporation, dilution; Southwick, 1984; Zimmerman, 1988; Búrquez & Corbet, 1991) and sources of unaccounted for variance among species (e.g. flower longevity and daily secretion rate; Devlin & Stephenson, 1985; Delph & Lively, 1992). Also, dynamic regulation of nectar offerings, by refilling nectar reservoirs after nectar has been removed or by readjusting the concentration of sugar as water evaporates, has been shown for several

species (e.g. Castellanos *et al.*, 2002 and references therein), and plant responses to intense nectar removal seem strongest for those pollinated by hummingbirds (reviewed by Ordano & Ornelas, 2004). The contribution of these factors to the variation in nectar traits cannot be comparatively evaluated because data on accumulated nectar is more widely and easily available for many species. In addition, the phylogenetic trees used for analyses included a large number of soft polytomies (see Appendix: Table A3) and employed arbitrary branch lengths. Therefore, the true amount of phylogenetic signal for these traits in these species is likely to be larger than indicated by the present analyses.

### Nonadaptive hypotheses

Among the nonadaptive explanations for the origin of nectar is the old idea that nectar is a by-product of a 'leaky phloem' and a 'sugar-excreting' mechanism (De la Barrera & Nobel, 2004); nectar could have originated as a leakage of the phloem solution resulting from the structural weakness of developing tissue exposed to the elevated hydrostatic pressure in the phloem (for experiments contra the position that NPR is in part a 'passive' result of phloem and xylem characteristics see Reed *et al.*, 1971 and Fahn, 1979). From a developmental perspective, the 'sugar excretion' hypothesis might also contribute to our understanding of why long-tubed flowers produce copious nectar (Table 5). On one hand, the surface area for the tubular portion of a flower changes in the same proportion as its length, e.g. a 10-cm-long flower would have a surface area four times as large as that for a 5-cm-long flower. Considering the relatively high transpiration rates of flowers (Galen *et al.*, 1999; Chapotin *et al.*, 2003), the amount of water loss through transpiration would be substantially higher for long-corolla flowers than for short-corolla flowers, leading to a greater accumulation of phloem-supplied solutes (mainly sugar) needing to be excreted for maintaining a relatively stable water potential in floral tissues. Corolla length may correlate with the storage capacity of nectar and particular solutes (e.g. amino acids and toxic compounds) that influence pollinator visitation. However, nectar itself does not explain how far the corolla tube can grow because it is an aqueous solution presented in glands in the flowers and often deeply concealed at the base of tubular flowers.

After nectar originated, subsequent evolution of nectar volume and its concentration might have been influenced by other factors, including the origin, size and anatomical structure of nectaries (Galetto & Bernardello, 2004 and references therein). This would then lead to a purely allometric relationship whereby scaling up the corolla tube also scales up the size of nectary tissue, and nectar characteristics can be constrained by allometric relationships between the size of the floral corolla tube and the size of the nectaries (Galetto & Bernardello,

2004). Whether or not these allometric associations are maintained by selection from pollinators or nectar robbers (Heinrich & Raven, 1972; Lara & Ornelas, 2001; Irwin *et al.*, 2004), and/or by plant allocation or by genetic correlations (Schemske & Bradshaw, 1999; Bradshaw & Schemske, 2003), is totally unknown. However, it must be remembered that allometric relations themselves can evolve in response to selection (Garland & Carter, 1994). Given that nectar extraction rates are a positive function of nectar volume and a negative function of corolla length, hummingbirds should prefer flowers with the highest nectar volumes and the shortest corollas because longer corollas make it harder for hummingbirds to get the nectar (Hainsworth, 1973; Montgomerie, 1984). Therefore, if flowers were evolving so as to enhance their attractiveness to hummingbirds, then we might expect to find a negative evolutionary correlation between nectar production and corolla length. Conversely, we found positive correlated evolution between corolla length and nectar production (Table 5), which is consistent with nonadaptive allometric expectations.

A plant–pollinator relationship based on predictions from one or two morphological or behavioural characters may be inappropriate and additional more complex multidimensional variables are needed to provide a more accurate description of evolutionary patterns. For example, Hurlbert *et al.* (1996) have suggested that flexible pedicels of *Impatiens capensis* flowers appear to facilitate hummingbird pollination (a ‘mobile’ pollination mechanism). Although a hummingbird’s efficiency to feed from flowers increases with increasing corolla diameter (Temeles, 1996) and that positive allometric relationships between corolla length and corolla entrance width have been reported for a number of plant species, some of which are hummingbird pollinated (e.g. Campbell, 1996; Temeles *et al.*, 2002), the occurrence of inverse relationships between lengths and widths in some hummingbird-pollinated genera indicates that plant species with long flowers do not always have wider flowers (Temeles *et al.*, 2002; see also Grant & Temeles, 1992). Furthermore, Castellanos *et al.* (2004) have shown experimentally in *Penstemon* species that hummingbirds removed almost twice as many pollen grains and efficiently extracted nectar from narrow flowers than from nonmodified flowers. They concluded that the narrow corollas of hummingbird-pollinated penstemons may function to improve the morphological fit of flowers to birds. All these evidences suggest that the positive correlation between corolla length and NPRs studied here may not result exclusively from floral allometry.

### Adaptive hypotheses

We found a positive association between nectar volume and corolla length, and between sugar production and corolla length after statistically controlling for covariation

related to phylogenetic inheritance (Tables 5 and 6). The strength of the relationships (ca.  $r = 0.4$ ) is perhaps remarkable considering the multifactorial nature of floral-trait evolution (see Stanton, 2003). Above, we note that simple geometry and allometry should yield a positive correlation between flower size (e.g. corolla length) and amount of nectar per flower. The simplest explanation for the significant relationships between NPRs and corolla length (i.e. allometry) do not necessarily mean that hummingbirds are actively choosing flower species on the basis of such relationships. These relations could arise either as a result of selection to maintain pollinator attractiveness or simply as a consequence of proximate allocation constraints (see Harder & Cruzan, 1990). As larger flowers and higher NPRs are both likely more costly to make (e.g. Pyke, 1991; Galen *et al.*, 1999), the correlations of corolla length and NPRs could be explained by variation in resource availability of different habitats. For instance, shifts into richer habitats could have allowed production of larger flowers and more nectar. However, differences in NPR values among habitat types (see Table 1 and Appendix: Table A2) are surely influenced by the inclusion of certain taxa inhabiting xeric conditions (a situation taken into account in our phylogenetic analyses) and the nonphylogenetic relationships between corolla length and NPRs still hold separately by habitat type (results not shown). The observed increase in corolla length and amount of sugar of hummingbird-pollinated flowers when compared with that in other flowers, along with other floral characters (e.g. Galen & Cuba, 2001; Castellanos *et al.*, 2004; Wilson *et al.*, 2004), is potentially associated with the evolution of hummingbird pollination. Although the relationship holds up within broad habitat type, NPRs can be very sensitive to microhabitat as well, sometimes varying several folds depending on conditions. One way to test that the association is due to selection by hummingbirds would be to analyse future data sets that also include plant species pollinated by other animals for comparison.

As predicted, we found a significant negative relation between sugar concentration and altitude (see also Stiles & Freeman, 1993) and between sugar production and altitude (Table 5), which suggests that nectar concentration in hummingbird flowers decreases to compensate for the higher viscosity at lower temperatures, and/or the result of differences in water and carbon resources, i.e. at high altitudes sugar production may be more costly. Stiles & Freeman (1993) observed that this negative relation is consistent with the fact that hummingbird-pollinated plants at high elevations are pollinated by smaller, less-energy demanding, short-billed trochiline species. However, the significant negative correlations between sugar concentration and altitude and between sugar production and altitude are removed when using multiple regression with PICs (Table 6). Although NPRs tend to decrease with increased altitude in some plant species (e.g. Cruden *et al.*, 1983), in some other

hummingbird-pollinated plant species no significant changes occurred along altitudinal gradients (e.g. Scobell & Scott, 2002). Also, it is not known whether nectar is more costly to produce and/or hummingbird-pollinated plant species are more resource limited at higher elevations than at lower elevations. Clearly, comparative studies of nectar production costs along altitudinal gradients are needed to tease apart among these hypotheses. Surprisingly, the commonly reported inverse relationship between volume and concentration in nectar was not found in our study (Table 5). Although hummingbirds choose concentrated sucrose solutions in preference to dilute ones (Van Riper, 1958; Stiles, 1976; Pyke & Waser, 1981; Roberts, 1996), experimental studies on sugar preferences have shown that they take nectar of a wide sugar concentration range (e.g. Martínez del Río *et al.*, 2001) and that hummingbirds do not prefer sucrose to hexose nectars, and even prefer hexose nectars at low concentrations (Fleming *et al.*, 2004). These findings suggest that preferences for different sugar compositions are concentration dependent (see also Lotz & Schondube, 2006).

The simplest scenario for pollinator-mediated selection on NPR is directional selection for high NPR. Once secreted, nectar can be manipulated in its concentration and constituents, and even reabsorbed to ensure visits by a given pollinator (or by more than one type of pollinator) and/or to recover some of the energy cost (e.g. Búrquez & Corbet, 1991; Nicolson, 1995; Nepi *et al.*, 2001; Ordano & Ornelas, 2004, 2005). But this scenario may be too simple (even including the cost of nectar), because plant fitness is a more complex function of pollinator behaviour. The best situation for the plant is to attract pollinators that bring conspecific pollen and remove their own pollen to carry it to conspecifics, in a pattern that produces matings of high fitness (e.g. that avoids self-pollination in many species) – all at minimal cost. What trait values achieve this, and the mechanisms of selection, may sometimes not be the ones that seem obvious at first thought (Campbell *et al.*, 1996; Hurlbert *et al.*, 1996; also Mitchell & Waser, 1992; Mitchell, 1993). Wilson *et al.* (2006) have postulated that for a shift from bee pollination toward hummingbird pollination to proceed among *Penstemon* species, there has to be evolution of the nectar characteristics of the plants. They emphasized that a penstemon must offer voluminous nectar, probably dilute and sucrose rich, to entrain hummingbird visitors. This first change during a shift from bee to bird pollination may be followed by changes in the floral dimensions, specifically from a globular to narrower corolla tubes that increase pollen transfer by hummingbirds (e.g. Castellanos *et al.*, 2003, 2004). Lastly, the evolution of floral traits needs to be understood in terms of all class of pollinators; plants may exhibit specializations for the most effective pollinators and in some cases for the less effective pollinators (Aigner, 2001). Differences in effectiveness certainly

can foster floral specialization to the most effective pollinator in some cases, but when adaptation to less effective pollinators requires little loss in the fitness contribution of a more effective pollinator, plants may specialize for the less effective pollinator (Aigner, 2001, 2004). The amount of nectar produced by hummingbird-pollinated plants has probably been under selection simultaneously exerted by multiple interactions with pollinating and nonpollinating fauna (Irwin *et al.*, 2004 and references therein). The strong positive relations between nectar traits and corolla length may have evolved in response to selection by pollinators. Yet, these same (or correlated) traits that lure pollinators may also increase the susceptibility of hummingbird-pollinated plants to antagonists (Grant & Temeles, 1992; Mitchell *et al.*, 1998; Lara & Ornelas, 2001; Castellanos *et al.*, 2004). We found a significant positive correlation between nectar concentration and corolla length in the conventional analysis but not in the phylogenetic analysis (see Table 5). The result from the phylogenetic analysis (Table 5) is inconsistent with the hypothesis posed by Bolten & Feinsinger (1978) that flowers with short-corolla tubes have low-concentrated nectar (dilute nectar) to deter nectar robbers (see also Pyke & Waser, 1981).

In summary, our results are consistent with the hypothesis of pollinator-mediated selection for larger flowers and high NPR (other things being equal). An alternative scenario is that pollinator preference has indeed led to the evolution of higher NPR but that the target of selection has been flower size, to which NPR is positively correlated phylogenetically.

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## Supplementary material

The following supplementary material is available for this article:

**Table A1** Reference list of the published studies included in the analysis sorted by alphabetical order.

**Table A2** Data on nectar volume and other floral traits of hummingbird-visited plants and original references.

**Table A3** The composite phylogeny of Angiosperm hummingbird-visited plants used to correct for the statistical nonindependence among species samples when assessing the relationship between nectar and corolla length and between nectar and altitude.

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