

# Relationship between corolla length and floral larceny in the South American hummingbird-pollinated *Campsidium valdivianum* (Bignoniaceae)

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Flowers with pink or red tubular corollas have been traditionally viewed as adapted for pollination by birds. However, tubular flowers frequently suffer illegitimate visits by nectar robbers. *Campsidium valdivianum* (Bignoniaceae) is an endemic hummingbird-pollinated climber with pinkish-red tubular flowers, which suffers high levels of floral larceny by a native bumblebee. We studied two populations of *C. valdivianum* in an Andean forest in South America and found that they differ significantly in their mean corolla length but not in their mean corolla width. The proportion of robbed flowers, as well as the mean number of holes per robbed flower was higher in the population with higher mean corolla length. Although robbing levels also differed between individuals within populations, corolla length was only significantly related to robbing frequency in the population with longest corollas. The positive relationship between corolla length and nectar robbing observed here might affect the fitness in this species and thus the direction and magnitude of selection of the floral trait.

Key words: *Bombus dahlbomii*, bumblebee, floral traits, nectar robbing, pollination, robbing frequency

## Introduction

Flowers with pink or red tubular corollas and high volumes of nectar have traditionally been viewed as adapted for pollination by birds, constituting a floral syndrome known as “ornithophily” (Faegri & Van der Pijl 1979). Under the selection pressure of long-billed pollinators, in

particular hummingbirds, long corollas are considered to be favored because this trait increases pollen transfer by excluding ineffective pollinators (Campbell *et al.* 1996, and references therein). However, in evolutionary terms, no trait is the result of one selective force, but rather represents a trade-off among different selective forces that may act in opposite directions. This

is the case with plants that need to attract and reward animals that effectively transfer gametes while avoiding nectar robbers (Irwin *et al.* 2001).

Nectar robbing is accomplished by making holes in a sympetalous corolla, allowing the flower visitor to obtain nectar “bypassing” the opening used by legitimate pollinators (Inouye 1980a). This behaviour is exhibited by some bird species (Navarro 1999, Lara & Ornelas 2001), carpenter bees (Waser 1979) and other bee species (Roubik 1982), and is particularly common among several species of bumblebees with short proboscides (Inouye 1980a, 1983, Hingston & McQuillan 1998, Maloof & Inouye 2000, Maloof 2001). Experimental studies have suggested that nectar robbers (short-billed hummingbirds) preferentially rob artificial flowers with long corollas (Lara & Ornelas 2001). This evidence is consistent with the hypothesis that nectar robbing is facultative (or behaviourally plastic) and appears when nectar(ies) cannot be reached from the opening of the tubular corollas.

Nectar robbing shows variation over space (Irwin & Maloof 2002), and part of this spatial variation might be attributable to variation in corolla length over space. Hingston and McQuillan (1998), comparing nectar robbing in *Epacris impressa* by *Bombus terrestris*, found that nectar robbing was more frequent in the population with the longest corollas. Nectar robbing is a widespread interaction, which has been studied in detail in some species of the northern hemisphere (for review see Maloof & Inouye 2000, Irwin *et al.* 2001, Irwin & Maloof 2002), nevertheless it has been almost completely ignored in the temperate forests of southern South America (Traveset *et al.* 1998), in spite of the unusually high incidence of biotically pollinated plants in these forests (Aizen & Ezcurra 1998).

*Campsidium valdivianum* (Bignoniaceae) is a climbing vine endemic to the understory of the temperate Valdivian rain forest of southern Argentina and Chile (Brion *et al.* 1988, Riveros *et al.* 1991, Smith-Ramírez 1993, Fraga *et al.* 1997), whose hummingbird-pollinated flowers (Wyatt 1983, Riveros *et al.* 1991) suffer high levels of nectar robbing by the long-tongued bumblebee *Bombus dahlbomii* (Apidae, Hymenoptera), the only native bumblebee in this

region (Abrahamovich & Díaz 2001). Secondary nectar robbery by butterflies (Pieridae, Lepidoptera) has been reported for populations in Chile (Riveros *et al.* 1991).

Floral length within the species is highly variable (Smith-Ramírez 1993), therefore we asked whether corolla length and the intensity of robbing vary between populations and within them. In this study we examined the corolla length, width, and floral larceny in two populations and explored the possible relationship between size and larceny.

## Material and methods

### The study system

*Campsidium valdivianum* is a climbing liana, with a strong, branched stem. It has flowers with long corollas, in comparison with those of other species in the study site, with hermaphroditic pinky-red tubular flowers pollinated by *Sephanoides sephaniodes* (Trochilidae) (Riveros *et al.* 1991, Smith-Ramírez 1993, Fraga *et al.* 1997), the only hummingbird species occurring in this forest type. Nectar standing crop has a volume of  $5.71 \pm 5.44 \mu\text{l}$  ( $n = 60$  flowers) and a sugar concentration of  $23.4\% \pm 2.9\%$  ( $n = 5$  individuals) dominated by sucrose (V. Chalcoff unpubl. data). The facultative nectar robber *Bombus dahlbomii* is the only native bumblebee in this region (Abrahamovich & Diaz 2001). It is very abundant and is known to pollinate many native plant species (Vázquez & Aizen 2003). The mean size of the proboscis of *B. dahlbomii* is 9.86 mm (Rebolledo *et al.* 2004). Although no data considering the length of the proboscis in comparison with the corolla length and width are available, it seems unlikely that the bumblebee legitimately visits *C. valdivianum*.

In Puerto Blest (41°S, 71°5'W, 760–856 m a.s.l.), flowering extends from September to December (Brion *et al.* 1988, Fraga *et al.* 1997) in the austral spring.

### Sampling methods

For this study, we established two sampling sites, Frias (population 1) and Cantaros (population 2),

ca. 2 km apart and similar in plant species composition and abundance of *C. valdivianum*.

In November 2001, during the peak of flowering of *C. valdivianum*, we randomly selected 20 flowering individuals at each site and collected 20 senescent flowers per individual ( $n = 800$  flowers). Flowers typically remain on the plant for ca. 20 days before falling off. As average height of flowering branches is ca. 7 m, we decided to sample flowers from the ground. This sampling measure permitted us to estimate the total number of holes per corolla suffered by each single flower during its lifespan. On the other hand this method underestimated absolute nectar robbing intensity because it did not allow us to assess whether robbers make a new hole each time they rob, or measure the secondary nectar robbing, which is beyond the scope of this paper. We chose plants growing at a minimum distance of 20 m from the nearest conspecific neighbour, to avoid pooling flowers belonging to different individuals.

We measured corolla length from the base to the top of the corolla (at the origin of the corolla lobes) and diameter with a hand calliper to the nearest mm and counted the number of holes made by nectar robbers. After falling down, corollas keep the original colour, shape and length for 1–2 days before beginning to wither and shrivel. Because we sampled fresh corollas only, we are confident that measures taken on senescent flowers resembled measures of flowers at the time when nectar robbing occurred. Nevertheless, this kind of measurement of corolla length may overestimate the actual distance that the flower visitor must reach in order to extract nectar from the bottom of the corolla tube, because accumulation of nectar in the flower may reduce this distance (Inouye 1980b).

## Data analysis

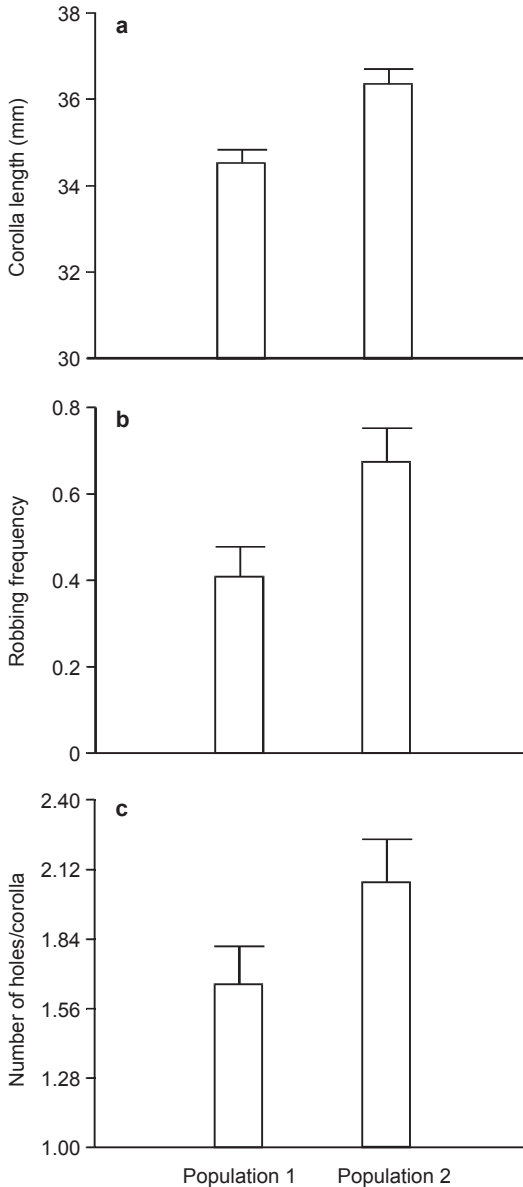
We calculated the individual corolla length and width, averaging the 20 flowers sampled per individual and estimated *robbing frequency* as the proportion of robbed flowers over total flowers sampled. We calculated the *number of holes per corolla*, averaging over all flowers sampled for each individual plant. We compared corolla

length and width, robbing frequency, and number of holes per corolla between populations with the *t*-test. Data on *number of holes per corolla* were  $\log_{10}$ -transformed in order to reach a normal distribution. Comparisons of mean corolla length among individuals within a site were performed using a one-way ANOVA on rank-transformed data due to heteroscedasticity of variance (Zar 1999). We then performed linear regression analysis to evaluate the influence of corolla length and width on the robbing frequency, and on the number of holes per corolla among and within populations. We considered individual plants rather than individual flowers as our sampling unit, in order to assure statistical independence. This approach was also preferred because we were interested in the evolutionary implications of variation among plants in corolla length and nectar robbing.

## Results

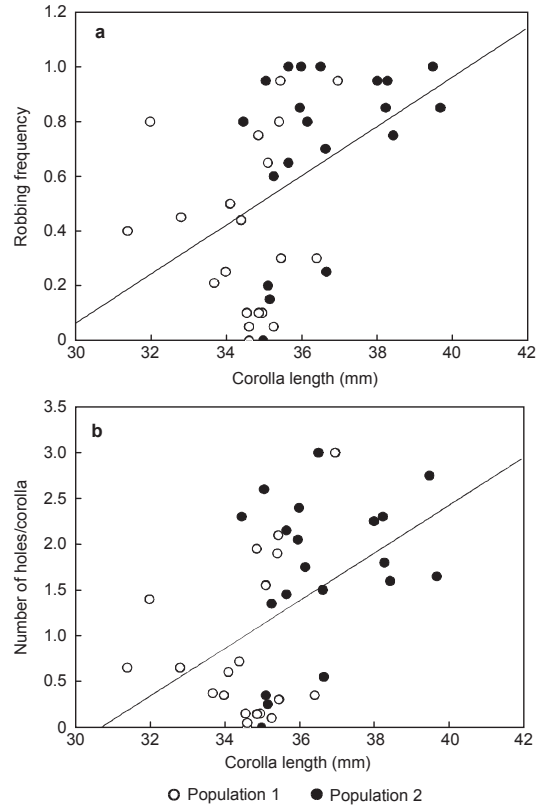
In *Campsidium valdivianum*, the mean individual corolla length ( $\pm$  S.D.) was  $35.6 \pm 1.8$  mm (Min = 31.4, Max = 39.7,  $n = 40$  plants) and the mean individual width ( $\pm$  S.D.) was  $7.01 \pm 0.43$  mm (Min = 6.02, Max = 7.86). The average corolla length in population 2 was significantly greater ( $P = 0.0002$ ) than that in population 1 (Fig. 1a). Within both populations, there were significant differences in average corolla length among individuals ( $F = 8.92$ ,  $P < 0.0001$  in population 1, and  $F = 8.91$ ,  $P < 0.0001$  in population 2). Although there were also significant differences among individuals in corolla width ( $F = 48.87$ ,  $P < 0.0001$  in population 1, and  $F = 6.01$ ,  $P < 0.0001$  in population 2), there were no differences between populations ( $t = 1.04$ ,  $P = 0.3062$ ).

Almost all individuals in both populations suffered some level of nectar robbing. We found robbed flowers in 19 out of 20 individuals in population 1 and in 18 out of 20 individuals in population 2. Robbing frequency (Fig. 1b) and number of holes per corolla (Fig. 1c) differed significantly between populations. On average, individual plants had higher robbing frequency ( $0.67 \pm 0.08$  in population 2, and  $0.41 \pm 0.07$  in population 1) and more holes per corolla ( $2.07$



**Fig. 1.** Comparison between two populations of *Campsidium valdivianum* in an Andean forest in South America. — **a:** Corolla length ( $t = 4.08$ ,  $P = 0.0002$ ). — **b:** Robbing frequency ( $t = 2.52$ ,  $P = 0.0160$ ). — **c:** Number of holes/corolla ( $t = 3.78$ ,  $P = 0.0008$ ). Data on number of holes/corolla were  $\log_{10}$ -transformed for the analysis. Error bars indicate  $\pm 1$  S.E.

$\pm 0.18$  in population 2, and  $1.66 \pm 0.15$  in population 1) in population 2 than in population 1. Considering all individuals, there was a highly significant positive linear relationship between corolla length and robbing frequency (Fig. 2a)



**Fig. 2.** Linear regression plots as a function of corolla length in flowers of *Campsidium valdivianum* in an Andean forest in South America. — **a:** Robbing frequency ( $r^2 = 0.19$ ,  $P = 0.005$ ,  $n = 40$ ). — **b:** Number of holes per corolla ( $r^2 = 0.15$ ,  $P = 0.014$ ,  $n = 40$ ).

and between corolla length and number of holes per corolla (Fig. 2b), suggesting an overall preference of nectar robbers for individuals with longer corollas. However, when we performed regression analysis within populations, we found only a significant relationship between corolla length and robbing frequency in population 2 ( $r^2 = 0.21$ ,  $P = 0.042$ ,  $n = 20$ ) and there were no significant relationships between corolla length and number of holes per corolla in both populations. No significant relationship between corolla width and nectar robbing was observed, either among or within populations (data not shown).

## Discussion

Trait variation is one of the key requirements of biological evolution (Darwin 1859). Due to these

variations, individuals within and among populations might suffer differential positive or negative selection promoted by their interactions with other organisms. In the present study, two close populations of *Campsidium valdivianum* significantly differed in their mean corolla length.

It has been largely recognised that floral traits are related to their animal visitors. In particular, it has been observed that corolla size is positively correlated with hummingbird (Campbell *et al.* 1991) and bumblebee (Galen 1989, Galen & Stanton 1989) visits together with pollination quality (Galen & Newport 1988, Galen 1989). Strikingly, we found that the population with higher mean corolla length was also preferentially robbed. This behaviour, with nectar being accessed either through the corolla throat or by piercing the corolla, as a response of variation of corolla length has been reported in comparisons among plant species (Lara & Ornelas 2001) or between populations of the same species (Hingston & McQuillan 1998).

Our results agree with those of Lara and Ornelas (2001), who found that short-billed hummingbirds preferentially robbed artificial flowers with longer corollas. In addition, our study shows that the number of holes per corolla also increased significantly with mean corolla length across individuals, suggesting that the population with longer corollas is not only robbed by preference, but also had a higher number of holes per corolla than the population with shorter corollas. In the regression analysis, we found a significant relationship between corolla length and both variables considering both populations together. However, when we analysed these relationships within populations, we found that only robbing frequency in population 2 was related to corolla length. The possibility exists that the size of populations allowed for detection of statistically significant differences in population 2 but not in population 1. Alternatively, the observed differences might be attributed to differences between populations. One possibility could be that there is a minimum length below which nectar robbing does not benefit the bees, therefore nectar robbers show a preference for the population with longer corollas and only within that population, they preferentially rob longer corollas. Accordingly, the populations

with shorter corollas, such as population 1 in our study, might be less preferred, and within them, robbers might exert less selection pressure. In line with this, Laras and Ornelas (2001) reported that the species with the longest corollas were preferentially robbed when compared with three different species with medium to long corollas. As seen in other communities (Irwin *et al.* 2001), another possibility could be that in population 1, the presence of plant species with similar flowers, that might effectively be visited by bumblebees, is providing a release from robbing. For example, *Berberis buxifolia*, *B. darwini* and *B. linearifolia* are frequently visited by *Bombus dahlbomii* (C. L. Morales unpubl. data), the bumblebee reported to rob nectar of *C. valdivianum* (Riveros *et al.* 1991). In line with this hypothesis, these plant species have the same flowering phenology as *C. valdivianum* and do occur in population 1 but not in population 2, hence they might be providing the release effect in the less-robbed population.

Finally, the spatial patterns of robbing may also be attributed to the spatial distribution of bumblebee nests (thus density) which change from year to year (D. Inouye pers. comm.). The fact that no differences were observed in corolla width between populations, together with the absence of any relationship between width and nectar robbing, suggests that this morphological trait might suffer less selection pressure in this hummingbird-pollinated species.

Our results are consistent with the hypothesis of robbing occurring when nectar cannot be reached via the throats of tubular flowers (Hingston & McQuillan 1998, and references therein) as is the case for *B. dahlbomii*. Nectar robbing might either allow robbers access to nectar in corollas that are too long to be visited in the "legitimate" way, or reduce the time they spend in flowers (Lara & Ornelas 2001) because the time spent by bumblebees visiting flowers may be positively correlated to corolla length (Inouye 1980b). A possible alternative explanation is that nectar robbers choose flowers with long corollas because they offer a more profitable reward for nectar robbers than other available flowers. In fact, studies have suggested that a positive relationship exists among the nectar volume, nectary and corolla size (e.g. Opler 1983). However,



preliminary results suggest that no correlation exists between corolla length and nectar volume (measured as standing crop) in this species ( $r = -0.05$ ,  $P = 0.69$ ,  $N = 60$ ).

Regardless of the mechanisms, our results show that the studied populations differ in their corolla length and are selectively visited by nectar robbers. It has been shown that floral larceny due to nectar robbing may negatively affect reproductive output (e.g. seed production) (Traveset et al. 1998, Irwin et al. 2001, Lara & Ornelas 2001, but see Maloof & Inouye 2000). Under the theoretical context of pollination syndromes (Faegri & Van der Pijl 1979) longer corollas may be viewed as a response that evolved from tight interaction between flowers and long-billed flower visitors. Therefore, the preferential nectar robbing of flowers with longer corollas observed here, raises the possibility of counter-selection on increasing length by nectar robbers (Lara & Ornelas 2001). In addition, it has been shown that hummingbirds avoid nectar-robbed flowers because they are nectar depleted (Irwin 2000). On the other hand, several studies document that robbers frequently pollinate the plants that they visit, hence they may have beneficial effects on the plants they rob (Maloof & Inouye 2000). In these cases, together with legitimate pollinators, robbers would select for longer corollas. A formal test of these hypotheses requires, first, examination of the effect of nectar robbing on fitness in this species, and second, evaluation of the relative importance of pollinators and nectar robbers on the net direction and magnitude of selection (Irwin et al. 2001) on this floral trait.

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