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Ornithophily in the Rhamnaceae: The pollination of the Chilean endemic *Colletia ulicina*

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Abstract

Colletia ulicina, an endemic species from the central region of Chile, displays red, tubular, scented, nectariferous flowers at the tip of its branches. Observations at two sites during two consecutive years indicate that the hummingbird *Sephanoides sephaniodes* (Trochilidae) visits the flowers on a regular basis. Captured hummingbirds had *C. ulicina* pollen loads on body parts matching the location of anthers and stigmas. The *C. ulicina*–*S. sephaniodes* interaction is well established (hummingbird visits were frequent, systematical, and occurred at different sites) although it lacks specificity (*S. sephaniodes* is a generalist hummingbird and *C. ulicina* has another, seasonally important pollinator, the even more generalist bumblebee *Bombus dahlbomii*). To the best of our knowledge this is the first documented report on bird pollination in the Rhamnaceae.

Keywords: Rhamnaceae; *Colletia ulicina*; Pollination; Ornithophily; *Sephanoides sephaniodes*

Introduction

Rhamnaceae constitute a medium-sized plant family (52 genera and ca. 925 species) in which pollination is generally effected by insects that visit the flowers for nectar and pollen (Medan and Schirarend, 2004). Most flower–visitor interactions are unspecialized, but as the floral tube varies in diameter and length across the family (Medan and Schirarend, 2004; Suessenguth, 1953), it has been suggested that flower tube depth is related to the composition and level of specialization of the flower visitor assemblage (Medan and Aagesen, 1995).

Within the tribe Colletieae (20 spp., mainly south American), relatively specialized pollinators like Hymenoptera and Lepidoptera prevail among long-tubed species, many of which have white or, at most, pinkish flowers. The trend to specialization was predicted to reach ornithophily in *Colletia ulicina* Gill. et Hook., a Chilean species in which the flowers, although not particularly large for the tribe, are narrowly tubular and bright red (Medan and Aagesen, 1995). Here we report observations and pollen load data that document the pollination of *C. ulicina* by hummingbirds (and, seasonally, also bumblebees) at two Chilean populations during two consecutive years. We also briefly characterize the plant-pollinator network to which the focal plant

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belongs, in an attempt to assess the relative importance that this mutualism has for the interacting partners.

Materials and methods

Colletia ulicina grows only in Chile, between 30°S and 36°S and from sea level to 1250 m a.s.l., on rocky soils, as spiny, decumbent shrubs that bloom from January to April (Tortosa, 1989). For general morphological information see Medan and Aagesen (1995) – flower, and Tortosa et al. (1996) – inflorescence. We worked at two populations (province Colchagua: near Las Peñas, 34°44′33″S 70°46′31″W, 720 m a.s.l., $n = 10$ individuals; province Talca: Natural Preserve Altos de Lircay, 35°36′21″S 71°04′21″W, 1215 m a.s.l., $n =$ ca. 100 individuals) (hereafter: LP and AL, respectively). Following a preparatory stay at LP in April 1998 field work was conducted in April 2000 (LP, AL) and February 2001(LP, AL).

To observe morphological details and to establish the timing of pollen presentation, stigmatic receptivity, and nectar secretion, we dissected ca. 25 fresh flowers under a stereomicroscope. Nectar was extracted with hand-drawn capillaries (which were kept for later calculation of nectar volumes), and its sugar content (as % sucrose equivalents) was determined with a hand refractometer modified for small volumes. Peroxidase activity in the stigmatic secretion was tested using the Peroxtesmo test (Dafni and Motte Maués, 1998).

To describe the visitor assemblage, flower visitors were observed, photographed and videotaped between 8:30 am and 8:00 pm (total observation time = 22 h). Representative insect individuals were collected with nets and mounted for later identification. Hummingbirds were mist-netted at AL and released after scrutiny of pollen loads. Hummingbird bill measures were taken on Chilean specimens kept at the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (Buenos Aires). To analyze pollen loads, pollen was extracted from visitors by rubbing on them (insects: mouthparts; sternal area and legs; hummingbirds: bill, forehead and throat) a small piece of glycerin jelly that was later melted on a microscopic slide. Co-flowering species were collected and identified; and their stigmatic loads (and those of *C. ulicina*) studied as follows: whole pistils were collected, air-dried and transported to the laboratory; each excised stigma was macerated for 24 h in a drop of 10% NaOH (w:v) on a slide. Then a small volume of melted glycerin jelly was added, and the stigma was gently squashed with a coverslip. Pollen tube growth was observed in *C. ulicina*: pistils fixed in FAA were soaked in 5% NaOH (w:v) at 30 °C, cleared in diluted NaClO, mounted in 0.1% decolorized aniline blue, and viewed with a fluorescence microscope.

Results

C. ulicina presents dense, spikelike inflorescences 5–50 cm long at the tip of the branches (Fig. 1). Each inflorescence displays 66.3 ± 30.9 (arithmetical mean ± S.D.) flowers per inflorescence ($n = 8$), of which 18.4 ± 11.7 are simultaneously active in pollen presentation and/or receipt ($n = 7$). Total number of flowers per individual was 20–5100 ($n = 8$). Flowers are hermaphrodite, with a coral-red, ca. 10 mm long floral tube 3.5 mm in diameter at its mouth. The five sepals are pink on their inner side, thus contrasting with the tube when the flower is open. The five anthers, alternate to the sepals, are somewhat versatile and introrse, and are located 2 mm below tube mouth and 3 mm above the stigma, leaving a narrow central passage (1 mm in diameter in newly open flowers, increasing in 2–3-day-old flowers up to 1.8 mm). A ring-like nectary is located near the base of the floral tube.

Presentation of pollen and receptive stigma started simultaneously upon flower aperture. A clear, sticky

Fig. 1. Inflorescence of *Colletia ulicina*. Bar = 1 cm (Photograph by D. Medan).

secretion covered the stigma and reacted positively to the peroxidase test. Flowers were mildly scented and secreted modest amounts of nectar (standing crop was $1.7 \pm 1.8\,\text{mm}^3$, $n = 11$; sugar concentration was $18.1 \pm 5.8\%$, $n = 12$).

One hummingbird species (*Sephanoides sephaniodes*), three apoid Hymenoptera (including the honeybee) and two butterflies visited legitimately the flowers (Table 1), but only the hummingbird and the bumblebee *Bombus dahlbomii* were frequent flower visitors, hence they probably effected most, if not all, pollination of *C. ulicina. S. sephaniodes* was regularly observed at both years in both field sites, while *B. dahlbomii* was only frequent at the AL site during the February 2001 field work.

The hummingbird's bill is longer than *C. ulicina*'s floral tube (15 ± 0.7 mm, $n = 10$). The bill can be easily introduced into the tube beyond anthers and stigma (which are located, respectively, 2 and 5 mm below tube mouth) because the bill is slender enough to reach at least 6 mm deep [bill diameter is 1.2 ± 0.09 mm ($n = 9$) at that distance from its tip]. At 6 mm insertion, reaching the nectary would demand a moderate (3–4 mm) tongue extension. Therefore, it can be safely assumed that contact between bill and anthers/stigmas occurred regularly. *B. dahlbomii* was observed reaching the bottom of the floral tube with its mouthparts, and the contact with reproductive structures was unavoidable.

C. ulicina plants received ca. four visits/h by the hummingbird (pooled data from both sites and years) and a similar number by the bumblebee (AL, February 2001 data only) (Table 1). Since visitation rate fluctuated along the day, a conservative extrapolation to a 12-h day indicates that an average plant would receive 30 daily visits from *S. sephaniodes* and a similar number by *B. dahlbomii.* When both visitors were present, their combined daily visits (amounting to ca. 1100 individual flowers contacted per individual) were enough for a medium-sized *C. ulicina* plant to have a large part of their active flowers contacted once a day. Because the bumblebee contacted almost twice the number of flowers per visit than the hummingbird did (24 vs. 13, Table 1), when sharing this resource with the hummingbird *B. dahlbomii* was involved in most (ca. 64%) contacts to individual flowers.

Most captured hummingbirds, and all captured bumblebees, carried *C. ulicina* pollen on their bodies (Table 1). Ninety percent of random-collected *C. ulicina* flowers carried stigmatic loads, which on average were rich (137 ± 107.0 pollen grains, $n = 120$ flowers from 10 individuals). In over one-third of the pollinated flowers at least one ovule (out of three present in the ovary) was penetrated by a pollen tube (36.7%, $n = 90$ flowers from nine individuals).

In addition to *C. ulicina*, at the AL site *S. sephaniodes* often visited *Fuchsia magellanica* (Oenotheraceae),

Table 1. Taxonomic affiliation and visitation behaviour of *Colletia ulicina* flower visitors

Flower visitor	*Sephanoides sephaniodes*	*Bombus dahlbomii*	*Butleria sexguttata*	*Neomaenas* cf. *fractifascia*	*Apis mellifera*	*Centris nigerrima*
Order	Apodiformes	Hymenoptera	Lepidoptera	Lepidoptera	Hymenoptera	Hymenoptera
Family	Trochilidae	Apidae	Hesperiidae	Nymphalidae	Apidae	Apidae
Field site	AL, LP	AL, LP[a]	AL	AL	AL	LP[a]
No. of recorded visits/ no. of plants involved	24/6	37/2	2/1	1/1	1/1	NR
Mean time between visits to same plant	14 min 30 s ($n = 17$)	14 min 6 s ($n = 30$)	NR	NR	NR	NR
Estimated no. of visits per plant per day	30	30	3–4	1	<1	NR
Mean visit duration (s)	51.9 ($n = 13$)	180 ($n = 12$)	NR	NR	NR	NR
Mean duration of contact with one flower (s)	1.34 ($n = 5$)	7.5 ($n = 12$)	NR	NR	NR	NR
Mean no. of flowers contacted per visit	13 ($n = 10$)	24 ($n = 12$)	<5	<2	NR	NR
Estimated no. of flowers contacted per plant per day	390	720	<20	<2	NR	NR
Percent visitor individuals bearing *C. ulicina* pollen	83.3 ($n = 6$)	100 ($n = 7$)	50 ($n = 2$)	0 ($n = 1$)	100 ($n = 1$)	100 ($n = 1$)

AL = Altos de Lircay, LP = Las Peñas, NR = not recorded.

[a] Visit inferred from pollen presence on a captured individual.

Ochagavia lindleyana (Bromeliaceae), and occasionally also *Mutisia decurrens* var. *patagonica* (Asteraceae). At the LP site the hummingbird visited *Lobelia tupa* (Lobeliaceae) in addition to *C. ulicina*. We recorded also visits by *B. dahlbomii* to *F. magellanica* at AL, and to *L. tupa* at LP. *C. ulicina* pollen was also recovered from most stigmas of *F. magellanica* and *O. lindleyana*, and pollen of these plant species was present on the bodies of both visitors and on stigmas of *C. ulicina*. Thus, a plant-flower visitor network existed at AL that involved as main mutualists *C. ulicina*, *F. magellanica*, *O. lindleyana*, *S. sephaniodes* and *B. dahlbomii.* At LP a more simple network included *C. ulicina*, *L. tupa, S. sephaniodes* and *B. dahlbomii*.

Discussion

Pollination of *C. ulicina* – the hummingbird component

Observations made at two different field sites in two consecutive flowering seasons indicate that the hummingbird *S. sephaniodes* is a regular and legitimate visitor of *C. ulicina* flowers, and strongly suggest that the hummingbird's feeding behavior causes interplant pollen transfer. The frequency of visits and their constancy across habitats and years indicate that the *C. ulicina–S. sephaniodes* interaction is a well-established mutualism. *Sephanoides sephaniodes* seems to be an effective pollinator of *C. ulicina* (and the only important one except when *Bombus dahlbomii* is also present) while *C. ulicina* was probably not the main food plant for *S. sephaniodes* at our study sites. This hummingbird is a well-known pollinator of many plants in the *Nothofagus*-dominated temperate forests of southern South America (ca. 20 genera, Aizen et al., 2002; Fraga et al., 1997; Urban, 1934; Willson et al., 1996).

This report validates speculations about ornithophily in *C. ulicina* (S. Vogel, pers. comm. to D.M. 1989; Medan and Aagesen, 1995) and first documents a case of bird pollination in the Rhamnaceae, a family in which only insect pollination was known to date (Medan and Schirarend, 2004). Several North American *Ceanothus* spp. (Rhamnaceae) have been included in lists of hummingbird food plants (e.g., *C. americanus* for the Ruby-throated Hummingbird *Archilochus colubris,* Coverstone et al., 2002) but whether or not true pollination mutualisms are implied in these interactions has still to be established.

The red, tubular, crowded, and well exposed flowers of *C. ulicina* are readily classified as ornithophilous (Proctor et al., 1996), also because of the diluted nectar and the relatively great separation between the nectar reservoir and the position of anthers and stigma. The presence of scent and the little amount of nectar per flower are untypical for the bird-pollination syndrome, however, and may reflect a compromise between conflicting selective pressures exerted by the bird- and the insect component of the visitor assemblage.

Pollination of *C. ulicina* – the insect component

As compared to other *Colletia* species with known visitor assemblages (*C. paradoxa*, D'Ambrogio and Medan, 1993; *C. spinosissima*, Basilio and Medan, 2001) the *C. ulicina* assemblage is striking because of its low diversity (only six spp. vs. 25 in *C. paradoxa* and 73 in *C. spinosissima*), the lack of relatively unspecialized visitors like Diptera and Coleoptera, and the presence of a vertebrate specialist. Large *Bombus* bees visit the flowers of *C. spinosissima* and were also observed on *Colletia hystrix* (D. Medan, unpublished), but the prominent role that *B. dahlbomii* can have in the pollination of *C. ulicina* is unprecedented among Colletias. *B. dahlbomii* is a native, generalist bumblebee widely distributed throughout southern South America (Abrahamovich et al., 2004) known to visit several species from at least seven plant families (Abrahamovich et al., 2001; Aizen and Ezcurra, 1998; Aizen et al., 2002). When it was frequent, this bumblebee behaved as the most active visitor to *C. ulicina*, and in comparison the remaining insect species in the assemblage (two butterflies and two apoid bees) seemed of marginal consequence to pollination.

B. dahlbomii was not observed during our April (austral Fall) field work. It is known that cloudy and rainy weather provides poor flight conditions for bees, while hummingbirds are less affected (Cruden, 1972). Although this needs confirmation, the role of *B. dahlbomii* as pollinator of *C. ulicina* would then be important during the summer months only. Another factor could further limit the significance of this bumblebee as pollinator: the relatively long visitation sequences that *B. dahlbomii* performs on single *C. ulicina* individuals probably cause geitonogamy, and under self-incompatibility this would lead to ineffective pollination. The breeding system of *C. ulicina* is still unknown, but the odds are high that it is self-incompatible like *C. paradoxa* and *C. spinosissima* (Basilio and Medan, 2001; D'Ambrogio and Medan, 1993;) and most Rhamnaceae studied to date (Medan and Schirarend, 2004).

S. sephanoides is a migratory species. Most populations leave for central Chile during fall, while a few are known to remain at the latitude of our field sites (C. Smith-Ramírez, pers. comm.). The pollination of *C. ulicina* during the last part of its flowering period thus seems to depend on resident hummingbirds that have this species as food source.

The plant-pollinator network and *C. ulicina* reproduction

A complete description of the plant-pollinator network to which *C. ulicina* belongs was beyond the scope of this paper, but we are confident that we managed to identify the most important mutualists associated to our focal plant (its pollinators and their alternative food plants in the community). It may not seem surprising that *C. ulicina* is shared as food plant by *S. sephaniodes* and *B. dahlbomii* (as is the case with the co-occurring plant species *F. magellanica* and *L. tupa*) since both animals are 'supergeneralist' pollinators. The fact that 'ornithophilous' flowers receive non-avian visitors (and that a non-avian mutualist at times functions as an important pollinator of *C. ulicina*) again confirms that the usefulness of pollination syndromes as predictors of assemblage composition is questionable (Pellmyr, 2002). Admittedly, sharing of food plants by *S. sephaniodes* and *B. dahlbomii* is not very common: in Argentina, across three sites studied by Vázquez (2002) in the Nahuel Huapi lake area (41°S) only three out of 15 plant species received visits from both the hummingbird and the bumblebee, and in a set of seven plant-pollinator webs located more to the North but also within the *Nothofagus* forests (39°S–41°S) there were only two plant species (out of 25) shared by *S. sephaniodes* and *B. dahlbomii* (M. Devoto, pers. comm.). West to the Andes, C. Smith-Ramírez (2005) found five shared species out of 26 from the Chiloé island flora (42°30′S).

The flowers of *C. ulicina* are homogamous, i.e., pollen and stigmas are presented at the same time, which allows both pollen export and receipt in a single pollinator visit, thus maximizing pollination efficiency (Medan and Basilio, 2001, where a discussion on homogamy in *Colletia* can be found). At our field sites the pollination service of *C. ulicina* seemed sufficient for fruit and seed set in terms of proportion of flowers that receive pollen, size of pollen loads, and pollen quality. Available data indicate that preemergent female reproductive success is comparable to that of other *Colletia* spp. (D. Medan, unpublished). Additional field studies are needed to gain better information on reproductive success (including seedling recruitment) of this species.

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References

Abrahamovich, A.H., Tellería, M.C., Díaz, N.B., 2001. *Bombus* species and their associated flora in Argentina. Bee World 82, 76–87.

Abrahamovich, A.H., Díaz, N.B., Morrone, J.J., 2004. Distributional patterns of Neotropical and Andean species of the genus *Bombus* (Hymenoptera: Apidae). Acta Zool. Mex. (ns) 20, 99–117.

Aizen, M.A., Ezcurra, C., 1998. High incidence of plant–animal mutualisms in the woody flora of the temperate forest of southern South America: biogeographical origin and present ecological significance. Ecología Austral. 8, 217–236.

Aizen, M.A., Vázquez, D.P., Smith-Ramírez, C., 2002. Natural history and conservation of plant–animal mutualisms in the temperate forest of southern South America. Rev. Chil. Hist. Nat. 75, 79–97.

Basilio, A.M., Medan, D., 2001. Pollinator assemblages of *Colletia spinosissima* (Rhamnaceae): composition, behavior, and specificity. Phyton (B. Aires) 2001, 129–139.

Coverstone, N., Stack, L.B., Witham, J., 2002. Understanding Ruby-Throated Hummingbirds and Enhancing their Habitat in Maine. Bulletin 7152. The University of Maine Cooperative Extension, Orono.

Cruden, R.W., 1972. Pollinators in high-elevation ecosystems: relative effectiveness of birds and bees. Science 176, 1439–1440.

Dafni, A., Motte Maués, M., 1998. A rapid and simple procedure to determine stigma receptivity. Sex. Plant Reprod. 11, 177–180.

D'Ambrogio, A., Medan, D., 1993. Comportamiento reproductivo de *Colletia paradoxa* (Rhamnaceae). Darwiniana 32, 1–14.

Fraga, R.M., Ruffini, A.E., Grigera, D., 1997. Interacciones entre el picaflor rubí *Sephanoides sephaniodes* y plantas del bosque subantártico en el Parque Nacional Nahuel Huapi, Argentina. Hornero 14, 224–234.

Medan, D., Aagesen, L., 1995. Comparative flower and fruit structure in the Colletieae (Rhamnaceae). Bot. Jahrb. Syst. 117, 531–564.

Medan, D., Basilio, A.M., 2001. Reproductive biology of *Colletia spinosissima* (Rhamnaceae) in Argentina. Plant Syst. Evol. 229, 79–89.

Medan, D., Schirarend, C., 2004. Rhamnaceae. In: Kubitzki, K. (Ed.), The Families and Genera of Vascular Plants. VI. Flowering Plants – Dicotyledons: Celastrales, Oxalidales, Rosales, Cornales, Ericales. Springer, Heidelberg, pp. 320–338.

Pellmyr, O., 2002. Pollination by animals. In: Herrera, C., Pellmyr, O. (Eds.), Plant–Animal Interactions. An evolutionary approach. Blackwell, Oxford, pp. 157–184.

Proctor, M., Yeo, P., Lack, A., 1996. The Natural History of Pollination. Timber Press, Portland.

Smith-Ramírez, C., Martínez, P., Núñez, M., González, C., Armesto, J.J., 2005. Diversity, flower visitation frequency and generalism of pollinators in temperate rain forests of Chiloé Island, Chile. Bot. J. Linn. Soc. 148, 399–416.

Suessenguth, K., 1953. Rhamnaceae. In: Engler, A., und Prantl, K. (Eds.), Die natürlichen Pflanzenfamilien, 2. Aufl. 20d. Duncker & Humblot, Berlin, pp. 7–173.

Tortosa, R.D., 1989. El género *Colletia* (Rhamnaceae). Parodiana 5, 279–332.

Tortosa, R.D., Aagesen, L., Tourn, G.M., 1996. Morphological studies in the tribe Colletieae (Rhamnaceae): analysis of architecture and inflorescences. Bot. J. Linn. Soc. 122, 353–367.

Urban, O., 1934. Botánica de las plantas endémicas de Chile. Soc. Imp. y Lit. "Concepción", Concepción.

Vázquez, D., 2002. Interactions among introduced ungulates, plants, and pollinators. A field study in the temperate forests of the southern Andes. Ph.D. Thesis, The University of Tennessee, Knoxville.

Willson, M.F., Smith-Ramírez, C., Sabag, C., Hernández, J.F., 1996. Plant–animal mutualisms in the temperate forests of Chile. In: Armesto, J.J., Villagrán, C., Kalin Arroyo, M. (Eds.), Ecología de los bosques nativos de Chile. Editorial Universitaria, Santiago, pp. 251–264.