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Mixed hummingbird–long-proboscid-fly pollination in 'ornithophilous' *Embothrium coccineum* (Proteaceae) along a rainfall gradient in Patagonia, Argentina

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Abstract The pollination ecology of eight populations of the tree *Embothrium coccineum* was studied along a steep rainfall gradient in NW Patagonia, Argentina. The showy red flowers suggest an ornithophilous pollination syndrome and they have been reported to attract hummingbirds in Argentina and hummingbirds and passerines in Chile. At each population, flower visitors were recorded and floral rewards were analysed. We found a highly significant increase in nectar concentration towards the drier end of the gradient, but this change was not related to the turnover of species in the flower-visitor assemblage of *E. coccineum*. In addition to the hummingbird *Sephanoides sephaniodes* (Green-Backed Firecrown, Trochilidae) which is widespread throughout the temperate forest at this latitude, other species seem to be locally important as pollinators of *E. coccineum* in some sites in Argentina (e.g. two long-tongued tanglewing flies (Nemestrinidae) of the genus *Trichophthalma*). The long-dated occurrence of tanglewing flies in South America, relative to the more modern hummingbirds, suggests that ornithophily may be a derived character in *E. coccineum*, the ancestral condition being pollination by Nemestrinidae.

Key words: *Embothrium*, hummingbird, Nemestrinidae, pollination, Proteaceae.

INTRODUCTION

In the past few years, evidence has accumulated on the many factors that affect patterns of interaction between plants and pollinators, both at the species and the community level. Variation in plant–pollinator interactions has been reported in response to differences in landscape structure (Steffan-Dewenter *et al.* 2002), habitat fragmentation (Aizen & Feinsinger 1994a,b), and changes in altitude (Arroyo *et al.* 1982; Malo & Baonza 2002; Medan *et al.* 2002), latitude (Elberling & Olesen 1999; Ollerton & Cranmer 2002) and insularity (Olesen & Jordano 2002). The effect of environmental variables on plant–pollinator systems can be approached by studying geographical gradients. These have been commonly used as ecological tools for understanding the influence of environmental factors on structure and functioning of terrestrial ecosystems (Vitousek & Matson 1991; Steffen *et al.* 1999), among other reasons, because geographical environmental variation gives rise to changes in the species composition of interacting guilds (Totland 1993; Medan *et al.* 2002; Fabbro & Körner 2004). Also, variability in the environmental conditions and the associated changes in the species composition of interacting guilds give rise to spatial mosaics of interactions which may be a key factor for speciation driven by interactions (Thompson 1998; Johnson & Steiner 1997; Totland 2001).

In the Patagonian region of South America, from 35° to 55°S, there is a strong east–west rainfall gradient caused by the Andes which impose an important barrier to the wet air masses from the Pacific Ocean (Paruelo *et al.* 1998a). This gradient is associated with a striking shift in vegetation in less than 150 km in an east–west direction from xeric desert shrubland to grass-shrub steppe, to a low stature tree cover and finally to closed canopy forest (Movia *et al.* 1982; Paruelo *et al.* 1998b; Austin & Sala 2002).

Two previous studies in the region analysed the community-scale effect of this geographical gradient on plant–pollinator interactions. Aizen and Ezcurra (1998) found a significant eastward decrease in bird-pollinated taxa throughout the region and related this pattern to a response of plants to abiotic conditions rather than to an innate scarcity of suitable bird flower-visitors. The ubiquity across the region of the native hummingbird *Sephanoides sephaniodes* (Molina) (Ralph 1985) further supported this view. An untested hypothesis, suggested by Aizen and Ezcurra (1998), is that bird-pollinated taxa might be unable to produce

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the high nectar rewards required for hummingbird pollination under water-stress conditions. Devoto *et al.* (2005) reported a significant eastward (i.e. from the forest towards the steppe environments) replacement in the flower-visitor assemblage of Diptera by Hymenoptera species. However, it is not known whether this community-level pattern would be reflected in the visitor assemblage of a given plant species with a widespread distribution along the gradient.

In this context, the present study analysed the effect of the rainfall gradient on the pollination ecology of a typically ornithophilous species (*Embothrium coccineum* J. R. Forst. & G. Forst. – Proteaceae) which is commonly visited by the hummingbird *Sephanoides sephaniodes* on both slopes of the Andes (Smith-Ramírez 1993; Fraga *et al.* 1997). The aims were to test whether (i) available nectar became less abundant and/or more concentrated towards the drier end of the gradient (as a response to increased water stress to plants and higher water evaporation from the nectar to the dry air); and (ii) the assemblage of flower-visitors to *E. coccineum* changed significantly along the rainfall gradient. Significant changes in the quantity/quality of nectar resources produced by *E. coccineum* and an associated absence of hummingbird pollination towards the drier sites would support Aizen and Ezcurra's (1998) hypothesis.

METHODS

Study populations and focal species

The study was carried out on the eastern slope of the Patagonian Andes within the Nahuel Huapi and Lanín National Parks in Argentina, approximately between 39–40°S and 71–72°W. The study sites encompassed humid *Nothofagus*-dominated forests (close to the Valdivian rainforests of Chile; Donoso Zegers 1993; Arroyo *et al.* 1996), to the easternmost outskirts of *Austrocedrus*-dominated dry forests on the border of the grass-shrub Patagonian steppe (Paruelo *et al.* 1998b). Within the described gradient, we selected eight sites (Table 1) ranging from 900 to 2550 mm in annual rainfall (a *c.* 2.8-fold change). The sites ranged in altitude from 727 to 1000 m a.s.l. and in mean annual temperature from 8 to 10°C (Movia *et al.* 1982). Average distance between populations was about 75 km.

Embothrium coccineum (locally known as 'notro' or 'ciruelillo') is a common tree species endemic to the temperate forests of southern South America (Sleumer 1984) which can reach 10 m in height (Sleumer 1984; Smith-Ramírez & Armesto 1998) but has a shrubby habit (1–4 m high) in the easternmost

Table 1. Geographic location, mean annual rainfall and altitude of eight populations of *Embothrium coccineum* studied in NW Patagonia, Argentina. Details on dates of field observations, no. of individuals sampled and estimated population sizes are also shown

Site (abbreviation)	Lago Queñi (LQ)	Paso Puyehue (PP)	Lago Tromen (LT)	Arroyo Pedregoso (AP)	Viuda de Barriga (VB)	Villa Traful (VT)	Lago Huechulafquen (LH)	Arroyo Minero (AM)
Date of field observations	Dec. 1999 Jan. 2000	Dec. 2001	Dec. 1998 Dec. 2003	Dec. 2001 Dec. 2002 Jan. 2003	Dec. 1997 Dec. 2003	Dec. 2001 Dec. 2002 Jan. 2003	Dec. 1997 Dec. 2003	Dec. 2001
Geographic coordinates	S 40°09′ W 71°43′	S 40°44′ W 71°53′	S 39°34′ W 71°26′	S 40°37′ W 71°35′	S 39°45′ W 71°22′	S 40°39′ W 71°21′	S 39°48′ W 71°12′	S 40°43′ W 71°05′
Mean annual precipitation (mm)	2550	2000	1750	1700	1450	1250	1050	900
Altitude (m.a.s.l.)	800	950	1000	870	970	900	780	727
Sample size (population size)	25 (100+)	10 (15)	25 (100+)	20 (50+)	15 (20)	10 (15)	200 (500+)	5 (10)

populations. The blooming period of *E. coccineum* extends from October to January. The showy red flowers suggest an ornithophilous pollination syndrome (e.g. Faegri & van der Pijl 1971; Proctor *et al.* 1996) and have been reported to attract a hummingbird (*Sephanoides sephaniodes* (Lesson) Trochilidae; Smith-Ramírez 1993; Fraga *et al.* 1997; Aizen *et al.* 2002) and other nectarivorous species of birds in Chile and Argentina (Smith-Ramírez & Armesto 1998, 2003). Reports of insect visitors to *E. coccineum* are scarce but include three unidentified species in Halictidae, Apidae and Vespidae (Puyehue; Riveros *et al.* 1991), and the colletid bee *Diphaglossa gayi* (Chiloé; Smith-Ramírez *et al.* 2005), both in Chile. Evidence suggests that *E. coccineum* is self-incompatible on both the western (Riveros, unpubl. 1991) and eastern (Devoto *et al.* unpubl. 1998) slopes of the Andes.

Flower visitors

At each population, 4–5 trained observers recorded flower-visitors to several full-bloom individuals of *E. coccineum* over a period of 7–8 days in mid-December. Additionally, a smaller team of 1–2 observers revisited most populations in mid-January and/or mid-December of a later year (see Table 1 for details). We recorded a given flower visitor as a putative pollinator only if it worked in such a way that pollen removal or deposition on stigma was possible.

Birds were identified visually. All visiting insects were collected and pinned for later identification. Placement, abundance and purity of pollen loads were characterized for all insect species with three or more recorded visits to flowers. To assess the presence of *E. coccineum* pollen, insect parts that showed visible loads (under 25× magnification) were rubbed with a small cube of gelatin-glycerin (Beattie 1971). Each cube was then melted on a slide and pollen grains of *E. coccineum* and other species were counted on 10 fields per slide (totaling a surface of 49.5 mm^2/slide).

Nectar production

To assess nectar production at each population, several flowering branches were enclosed in paper bags during 1 day, and the next morning the nectar volume was measured using a 10-μL Hamilton syringe (accuracy: ±1% of nominal volume). We also measured equivalent sucrose concentration (in °Brix: g of solute per 100 g of solution; Bolten *et al.* 1979) using a hand-held refractometer modified for small volumes. The length of the flower tube was measured to the nearest 0.1 cm at each site.

Quality of the data set

Species turnover in pollinator communities can be very significant over time and space (Williams *et al.* 2001; Potts *et al.* 2003; Herrera 2005). Assemblage spatial variability was accounted for by sampling in several sites encompassing much of the regional environmental variation. The absence of *E. coccineum* eastward from our driest site (authors' pers. obs. 1997 & 2003) suggests that samplings included the total longitudinal distribution of this species, at least on the eastern slopes of the Andes. Regarding seasonal variation, we believe that the relatively short sampling window we used did not seriously flaw our data given the unimodal and strongly seasonal reproductive phenology of the populations studied, where the activity of most pollinators is markedly concentrated in December and, to a lesser extent, in January (Smith-Ramírez & Armesto 1994; Riveros & Smith-Ramírez 1996). A third concern is that our survey may undersample interannual variation. We hope to have partially circumvented this problem by sampling five out of eight sites in at least two different seasons (Table 1).

Data analysis

A log-linear analysis was performed with sites and frequencies of species of flower visitors (grouped in four categories: hummingbirds, bees, flies and other insect orders) as response variables to test for overall differences among sites in flower-visitor composition. Linear regressions were performed to relate variations in floral characters to rainfall changes along the gradient. In order to achieve normality and homoscedasticity, nectar volume was natural-log transformed, whereas the arcsine square-root transformation was applied to sucrose concentration. Means are reported with standard errors unless otherwise noted.

RESULTS

Flower visitors

In seven out of the eight populations surveyed, insects and/or birds visiting flowers of *E. coccineum* were recorded (Table 2). We recorded no visitors to *E. coccineum* at Villa Traful, although observations there could only be made on few, rather isolated individuals within clearings of a closed forest. The composition of the visitor assemblage showed a significant variation between sites (group-level analysis; $\chi^2 = 19.79$, $P = 0.0030$). However, this species diversity was not significantly related to changes in annual rainfall along the gradient (Spearman rank-order

Table 2. Species diversity of visitors and most common pollinators of *Embothrium coccineum* at seven populations in NW Patagonia, Argentina

Sites	LQ	PP	LT	AP	VB	LH	AM
Visitor group							
Birds	1	1	2	2		4	
Hymenoptera	5	1	4	1		3	4
Diptera	8		4		2		
Coleoptera + Lepidoptera	3						
Total species richness of the visitor fauna at each population	17	2	10	3	2	7	4
Most common pollinators							
Sephanoides sephaniodes							
Trichophthalma niveibarbis			4		10		
Trichophthalma philippii	5		3		2		
Corynura prothysteres			1			1	4
Corynura sp. 2				12			

For the common pollinators, a shaded cell indicates that the (morpho)species (rows) was observed visiting flowers of *E. coccineum* at the corresponding population (columns). The number of individuals of each species caught visiting *E. coccineum* is also provided. Given their high abundance in the field, individuals of *Trichophthalma niveibarbis* and *Corynura prothysteres* were only captured for the reference collections. Birds were not captured. AM, Arroyo Minero; AP, Arroyo Pedregoso; LH, Lago Huechulafquen; LQ, Lago Queñi; LT, Lago Tromen; PP; Paso Puyehue; VB, Viuda de Barriga.

Table 3. Proportion of pollen grains from *Embothrium coccineum* in pollen loads carried by the main insect visitors to flowers of *E. coccineum* in Patagonia, Argentina

Species	Proportion of grains from *E. coccineum* in the pollen load (mean ± SD) and (*n* of loads examined)	Placement of load on insect's body
Corynura sp. 2	0.69 ± 0.28 (11)	Hindlegs, metapleura
Corynura prothysteres	0.87 ± 0.18 (3)	Ventral part of metasoma, hindlegs
Trichophthalma niveibarbis	0.77 ± 0.20 (7)	Ventral part of metasoma
Trichophthalma philippii	0.91 ± 0.08 (5)	Ventral part of metasoma

correlations; hummingbirds, $\rho = 0.63$, $P = 0.12$; Hymenoptera, $\rho = 0.21$, $P = 0.63$; Diptera, $\rho = 0.43$, $P = 0.33$; other orders, $\rho = 0.61$, $P = 0.14$). Overall species richness of visitors to the flowers of *E. coccineum* was not related to changes in rainfall (Pearson Correlation Coefficient, $r = 0.65$, $P = 0.15$).

Overall, 32 flower-visitor species were recorded. However, we focused on five species that were recorded six or more times in the study (Table 2). These were the hummingbird *Sephanoides sephaniodes* (observed at five populations), two halictid bees (*Corynura prothysteres* (Vachal) at Lago Tromen (LT), Lago Huechulafquen (LH) and Arroyo Minero, and *Corynura* sp. 2 at Arroyo Pedregoso (AP)), and two nemestrinid flies (*Trichophthalma niveibarbis* (Bigot) at LT and Viuda de Barriga (VB), and *T. philippii* Rondani at Lago Queñi (LQ), LT and VB). Previous reports (Fraga *et al.* 1997; Smith-Ramírez & Armesto 1998; Aizen *et al.* 2002) also identified the nectarivorous *Sephanoides sephaniodes* as a possible pollinator. We recorded the two halictids collecting only pollen. They had on their bodies a consistently high proportion of pollen grains from *Embothrium* (69–87%; Table 3). Both species of nemestrinids (tanglewing flies), firmly grasped petal lobes of *E. coccineum* as they foraged for nectar and while doing so they touched the tip of the pollen presenter, where the stigmatic slit is located, with their often pollen-loaded abdomen (Fig. 1). The mean proportion of pollen grains from *Embothrium* on their bodies was 77% to 91% (Table 3). Purity of pollen loads of trapped *Trichophthalma* individuals was variable on *T. niveibarbis* but high on *T. philippi* (Table 3). These flies' proboscides were 7.27 ± 0.32 mm ($n = 13$) and 8.88 ± 0.33 mm ($n = 9$ individuals) long respectively.

In one site (AP), a rare hummingbird species (*Oreotrochilus leucopleurus* Gould; Trochilidae) was recorded foraging for nectar on *Embothrium*. At several sites, other birds (*Phrygilus patagonicus* Lowe, *Phrygilus gayi* (Gervais) and *Enicognathus ferrugineus* (Müller)) were seen feeding on flowers but severely damaging them in the process. Among insects, seven Diptera, 12 Hymenoptera, two Coleoptera and one Lepidoptera (Table 2) were also recorded, but were

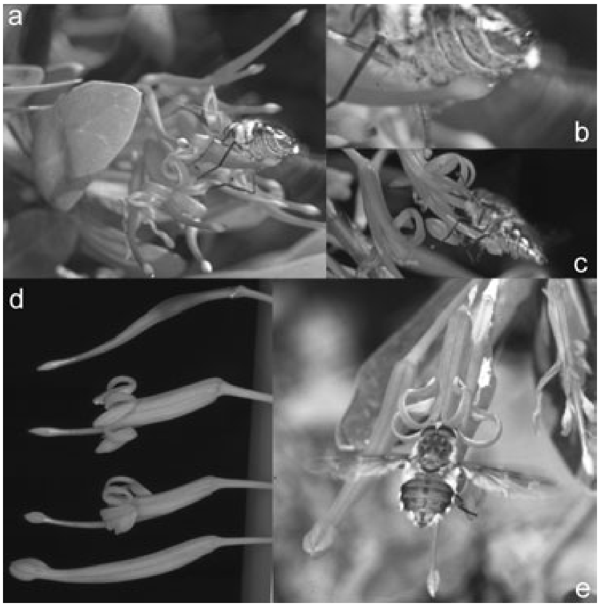

Fig. 1. The tanglewing fly *Trichophthalma niveibarbis* (Nemestrinidae) feeding on flowers of *Embothrium coccineum* (a,e); details of *T. niveibarbis* touching stigmas of *E. coccineum* while foraging (b,c); flower stages of *E. coccineum* (from bottom to top): bud, male phase (notice secondary pollen presentation on the swollen tissue surrounding the stigma), female phase, wilting (corolla abscised) (d).

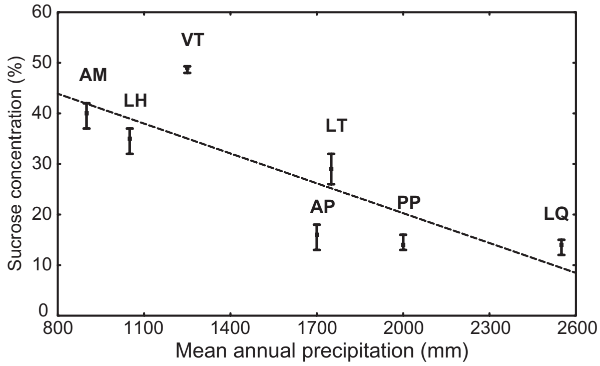

Fig. 2. Nectar sucrose concentration in flowers of E. coccineum at seven populations of NW Patagonia, Argentina. No data were available for Viuda de Barriga populations. AM, Arroyo Minero; AP, Arroyo Pedregoso; LH, Lago Huechulafquen; LQ, Lago Queñi; LT, Lago Tromen; PP; Paso Puyehue; VT, Villa Traful.

disregarded as pollinators either because they seldom touched reproductive structures during their foraging activity (e.g. *Butleria quilla* (Evans)), they were very rarely recorded on flowers (e.g. Ichneumonidae sp.) or they carried pollen tightly packed in their corbiculae (e.g. *Cadeguala occidentalis* (Haliday)) thus rendering pollination unlikely. At LQ, LT and Villa Traful *Elaenia albiceps* ((D'Orbigny & Lafresnaye), Tyrannidae) was recorded eating fruits of several species, although not foraging on flowers of *E. coccineum*, even though this species has been reported as a frequent pollinator of *E. coccineum* at Chiloé, Chile (Smith-Ramírez & Armesto 1998).

Flower phenology and reward

The flowers of *E. coccineum* remained open from 3 to 5 days, had a mean (range) tube length of 14.06 mm (10.53–18.96; $n = 103$) and a mean (range) nectar volume of 3.35 µL (2.34–3.96; $n = 124$) with a sucrose concentration of 30% (6–62; $n = 107$). Nectar volume did not show any consistent change across the rainfall gradient ($R^2 = 0.14$, $P = 0.40$). However, sucrose concentration of nectar significantly increased towards the drier sites ($R^2 = 0.67$, $F_{1,6} = 10.4$, $P = 0.023$; Fig. 2).

We compared our results with data from a population of *E. coccineum* at Isla Grande de Chiloé, Chile (42°30′S 73°35′W; rainfall: 2178 mm; Smith-Ramírez 1993). In all our populations, nectar volume was significantly lower than in Chiloé (mean: 15.6 µL, SD: 11.8, $n = 69$; $P < 0.05$ in all seven Tukey's HSD tests). Sugar concentration at Chiloé (mean:10.3, SD: 5.5, $n = 23$) resulted significantly lower than in all our populations ($P < 0.05$ in all seven Tukey's HSD tests).

DISCUSSION

Nectar sugar concentration increased eastwards with decreasing rainfall (Fig. 2), but nectar volume and flower-tube length showed no geographical trends. A possible explanation for this is that increased water evaporation in the drier populations might stimulate additional nectar secretion (which *E. coccineum* is capable of; Smith-Ramírez & Armesto 1998) leading to a progressive increase in sugar concentration inside the floral tube (Corbet *et al.* 1979; Nicolson & Van Wyk 1998). At the same time, we recorded across the gradient a changing assemblage of flower visitors to *E. coccineum*. The hummingbird *Sephanoides sephaniodes* and four insect species (*Trichophthalma niveibarbis*, *T. philippii*, *Corynura prothysteres* and *Corynura* sp. 2, were the most common pollinators. Tanglewing flies and hummingbirds were not recorded as floral visitors at the two driest sites, perhaps because of the high viscosity of the sucrose-rich nectar at these populations. However, our analysis suggests that variations in the flower-visitor assemblage of *E. coccineum* are independent of the qualitative change in its nectar rewards. The particular assemblage that visits *E. coccineum* at a given site is a non-random 'choice' of the pollinator pool available at that point of the rainfall gradient. The entire pollinator community significantly changes across the gradient 'offering' fewer flies and more bees towards the drier end (Devoto *et al*. 2005). Summarizing, the rainfall gradient influences reward quality apparently through a response of plants to abiotic stress (as suggested by Aizen & Ezcurra 1998) but for *E. coccineum* reward changes seem uncoupled with assemblage changes (contrary to Aizen & Ezcurra 1998).

Aizen *et al*. (2002) suggested that the apparent lack of visitation of the passerine *Elaenia albiceps* to *E. coccineum* outside the island of Chiloé in Chile might be related to the higher sucrose/hexoses ratio in the nectar of *Embothrium* on the eastern and western slopes of the Andes as compared with that of Chiloé (Smith-Ramírez & Armesto 1998). Our results suggest this behaviour might also be a consequence of the lower nectar volumes available per flower on the eastern slope of the Andes as compared to Chiloé. The high feeding plasticity of *E. albiceps* (Smith-Ramírez & Armesto 1998) may allow it to change its preference towards better-rewarding resources. Additional data on the nectar features of *E. coccineum* from the western slope of the Andes might prove valuable in this regard.

The data we present here are consistent with previous reports (mentioned above) that *S. sephaniodes* is a major pollinator of *E. coccineum* on both slopes of the Andes because of its widespread populations and foraging behaviour. This well-studied pollinator has a bill which is long enough (15.58 ± 0.15 mm; $n = 29$; Fraga *et al*. 1997) to reach the base of the floral tube (14.06 mm) and has been reported to transport large loads of pollen of *E. coccineum* on its body (Fraga *et al*. 1997; Smith-Ramírez & Armesto 1998). The presence of additional bees and tanglewing flies (Nemestrinidae) in the visitor assemblage suggests a mixed bird-insect pollination mechanism for *E. coccineum*. Several papers report similar cases of typically ornithophilous species (Pleasant & Waser 1985; Macior 1986; Mayfield *et al*. 2001; Díaz & Cocucci 2003; Medan & Montaldo 2005; Robertson *et al*. 2005) where the expected pollinator (a hummingbird) can be outperformed as pollinator by a 'morphologically unfitting' visitor (e.g. a bumblebee) in terms of outcross pollen deposited on stigmas or seed production per visit.

The remarkably close fit between the morphology of tanglewing flies and *E. coccineum* is perplexing given the ornithophilous 'pollination syndrome' of the red tubular flowers of *E. coccineum*. Pollination by tanglewing flies deserves special attention considering that this family is rather primitive among Diptera (Willemstein 1987; Mostovski & Martinez Delclos 2000), and that pollination by this group of flies is a most unusual phenomenon. It has been reported only in southern Africa (Manning & Golblatt 1997) and southern South America (Angulo 1971; Aizen *et al*. 2002; Devoto & Medan 2006), but the biology of tanglewing flies remains widely unknown (Peña 1996). In southern Africa, there is a particular guild of plant species with long-tubed flowers for which the long-proboscid tanglewing flies remain the only true (and highly specialized) pollinators (Goldblatt & Manning 2000). These highly specialized interactions often lead to an increased selective pressure on certain floral traits, such as selection for longer spurs in populations of *Disa* orchids exerted by the tanglewing fly *Moegistorynchus longirostris* (Johnson & Steiner 1997). However, the nectar sugar concentration of typical 'Nemestrinidae flowers' (20–30% sucrose equivalents; Manning 2004) and that of typical 'hummingbird flowers' (*c*. 20%; Bolten & Feinsinger 1978) are similar and both resemble the features of nectar from *E. coccineum* (26%). This suggests that in the temperate forests of southern South America, hummingbirds and tanglewing flies likely constitute a true 'functional group' of pollinators (*sensu* Fenster *et al*. 2004) that would be exerting a coincident selective pressure, at least concerning nectar sugar concentration. In fact, the historical coexistence of tanglewing flies, which differentiated in South America through the Cretaceous period (Bernardi 1973), with *Embothrium*, which is present in South America at least since the Oligocene (Dusén 1899; Prance & Plana 1998), and the much later appearance of hummingbirds in the early Miocene in Andean Patagonia (Bleiweiss 1998) suggest that ornithophily in *E. coccineum* may be a recent acquisition. The ancestral condition was probably pollination by Nemestrinidae (but see Aizen & Ezcurra 1998). Interestingly, a comparative survey of modes of pollination within various plant genera (Grant & Grant 1968) suggested strongly that in the Western North American flora, hummingbird flowers are derived from bee flowers in numerous independent phyletic lines. Admittedly, the evidence presented in this paper in favour of the importance of tanglewing flies as pollinators of *E. coccineum* still needs to be evaluated through careful field experiments.

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