RESEARCH PAPER

Pollination ecology of *Disterigma stereophyllum* (Ericaceae) in south-western Colombia

L. Navarro¹, P. Guitiáñ² & G. Ayensa¹

¹ Departamento de Biología Vegetal, Facultad de Ciencias, Universidad de Vigo, Vigo, Spain
² Departamento de Botánica, Facultad de Farmacia, Universidad de Santiago de Compostela, Santiago de Compostela, Spain

INTRODUCTION

In the understorey of neotropical forests, hummingbird pollination is as frequent as that by medium to large bees (Bawa 1990). Although species of Ericaceae are prominent in tropical montane forests throughout the world, surprisingly little is known about their biology and ecology (Luteyn 1989). There have been relatively few studies of the breeding systems and pollination biology of neotropical Ericaceae (Melampy 1987; Murray et al. 1987; Murcia & Feinsinger 1996; Navarro 1999, 2001; Busby 2000; Kramer 2001; Navarro et al. 2007). Within this family, many species show floral traits apparently adapted to hummingbird pollination (i.e. brightly coloured, long tubular flowers with inferior ovaries and abundant dilute nectar).

Several studies of Ericaceae in Colombian montane forest have shown that species of this type are indeed mainly pollinated by hummingbirds (Snow & Snow 1980; Navarro 1999; Busby 2000). Other neotropical ericads show floral traits apparently adapted to bee pollination (i.e. shorter, urceolate, white or light-coloured corollas) but, in spite of the commonness of such ‘bee pollination syndrome’ species, their pollination has received little attention (although see Snow & Snow 1980; Busby 2000).

Within the genus *Disterigma* there are species that appear to conform to the bee pollination syndrome, hummingbird pollination syndrome, or both. However, floral characteristics do not always correspond well to the observed pollinator spectrum. For example, *Disterigma alaternoides* has small, white flowers that produce small...
amounts of concentrated nectar, suggesting mellitophily (bee pollination), but other floral traits such as rigid anthers attached to the outside of a nectariferous disk forming a tunnel leading to the nectar, inferior ovaries and scheduling of pollen delivery, suggest ornithophily (Snow & Snow 1980; see Thomson et al. 2000 for traits assigned to these pollination syndromes). In spite of these mixed characteristics, the species is reported to be entirely bee-pollinated in the Colombian Andes (Snow & Snow 1980). This example exemplifies the fact that whereas pollination syndromes (sensu Faegri & van der Pijl 1979) apply to some specific cases (e.g. Hargreaves et al. 2004; Wilson et al. 2006), many other studies of single species have failed to match the putative syndrome with predicted major pollinators (Ollerton et al. 2003; Zhang et al. 2005; Valdivia & Niemeyer 2006).

In the present study, we investigated the pollination ecology of the neotropical species *Disterigma stereophyllum* with the aim of assessing whether floral traits match its usual pollinator fauna. *D. stereophyllum* inhabits neotropical montane forest, with a distribution restricted to southern Colombia and northern Ecuador, and exhibits a mix of floral characteristics that suggests adaptation for pollination by both hummingbirds and bees (Navarro et al. 2007). Traits suggesting adaptation for hummingbirds include copious amounts of dilute nectar and the manner of pollen presentation, whereas the short, white, and urceolate corolla, together with its erect orientation suggest bee visitation.

**STUDY AREA**

The study was carried out in the La Planada Nature Reserve, near the village of Ricaurte (Nariño Department, Colombia) (1°10′ N, 77°58′ W) during March–April 1998. The reserve is located on the western flank of the Andes and comprises 3200 ha of montane wet forest (bmh-PM in the classification of Holdridge 1996), at altitudes ranging from 1200 to 2100 m above sea level. Mean annual precipitation is 4375 mm and temperature ranges between 12 and 23 °C. More information on the study area can be found in Orejuela (1987) and Restrepo & Gómez (1998).

**PLANT NATURAL HISTORY**

*Disterigma stereophyllum* (A. C. Sm.) Luteyn (Ericaceae) is an epiphyte. In the study area it typically occurs in fringe communities around mature forest. It has several flowering peaks over the year. Flowers are hermaphrodite, with a 9-mm long fleshy, white urceolate corolla with small purple lobes. Each flower has on average 362 ovules (ranging among 252–501). Flowers are protandrous and open for 4 days. The style is slightly exerted and extends beyond the anthers (Fig. 1). On average, flowers produce 48 µl of nectar per day, with a low-sugar concentration (21.2 ± 6.6% w/w), and, except for pollen, this species did not present any other floral reward. The species is cryptically self-incompatible (Navarro et al. 2007). The fruit, a translucent white berry when mature, is eaten by frugivorous birds (the golden tanager *Tangara arthus* and the orange-bellied euphonia *Euphonia xanthogaster*; L. Navarro, unpublished results). Voucher specimens of *D. stereophyllum* from the study site are deposited at the herbarium of the Universidad de Pasto (Nariño Department, Colombia).

**METHODS**

**Standing crop of nectar**

On three different days, we measured the amount of nectar available to visitors in the same areas in which we did pollinator censuses (see below). Just before each pollinator census, at 6:30, 8:30, 10:30, 12:30, 14:30 and 16:30 hours, we selected 10 flowers at random and extracted nectar with micropipettes. Sugar concentration in nectar was measured with a pocket refractometer. All measurements were made during dry weather.
Floral visitation

We censused flower visitors in two standard adjacent patches of plants of no more than 20 m² area, each of which could be easily observed from a distance of about 4 m. All plants were at a height of <3 m. Both areas formed part of fringe communities around mature forest. The number of flowers ranged between 120 and 150 per patch during the censuses. We tallied visits during the month of March in a series of 60-min censuses at different times of the day, totalling 66 h, with equal effort at different times of day. For each visit, we recorded the species of visitor and the number of flowers probed. We also recorded type of visit (nectar extraction or primary nectar robbery if the visitor made a hole in the corolla tube; Inouye 1983). All visitors were identified with the aid of both field guides (Hilty & Brown 1986) and collections deposited at La Planada Nature Reserve.

Pollinator efficiency on pollen deposition

The day before each census, we bagged a number of virgin flowers on two or three plants. During each census, these flowers were unbagged, and, after a single visit, each pistil was collected to count the number of pollen grains deposited on the stigma by the visitor in question.

Nectar robbing

We examined a total of 1106 flowers from more than 70 plants across the flowering season, recording whether or not each flower had been robbed. Field determination of whether a flower has been robbed is straightforward, as the robber leaves a clearly visible incision in the corolla.

Data analysis

We used analysis of variance to compare nectar standing crops (volume and concentration) over time, the number of flowers visited by each visitor on arrival, and the number of pollen grains found on the stigma after each visit. All proportional data were arcsine-square root transformed before ANOVA. Number of pollen grains was transformed by square root (x + 0.5). All analyses were carried out with the statistical package Systat (1997). In the text, mean values are given with their standard errors.

RESULTS

Nectar properties

Nectar standing crop showed apparent alternation between observation periods (Fig. 2a), but analysis of variance did not reveal any significant among-period variation (F$_{5,54}$ = 1.9; P = 0.1019). Sugar concentration of nectar varied significantly over the day (F$_{5,42}$ = 4.3; P = 0.0030): a posteriori Tukey tests showed that concentration was significantly lower in the early morning than at midday, and lower in the late afternoon than at midday (Fig. 2b).

Floral visitation

_Disterigma stereophyllum_ was one of the most abundant nectar sources for nectarivorous birds at La Planada during the study period (L. Navarro, unpublished results). Flowers of _D. stereophyllum_ were visited by a diverse guild of visitors (Table 1). Of the 4178 recorded visits to individual flowers, 93.9% were legitimate nectar extraction visits, while the remaining 6.1% were visits by primary nectar robbers (in all cases the white-sided flower-piercer _Diglossa albilatera_). We observed no secondary nectar-robbing visits. Thus, the ratio of legitimate visits to nectar-robbing visits was 15.4:1.

The most frequent legitimate visitor was the booted racket-tail (_Ocreatus underwoodii_), accounting for 49.5% of total visits, followed by the blue-tailed emerald (_Chlorostilbon mellisugus_), accounting for 24.8% of visits. Both of these hummingbird species have territorial behaviour, defending patches from visits by other hummingbirds. There was significant variation among pollinators in the...
number of flowers visited per legitimate visit ($F_{9,2042} = 11.7$, $P < 0.0001$). Tukey tests indicated that this variation was due to the high number of flowers visited per visit by the green-fronted lancebill ($Doryfera ludoviciae$) and bumblebees ($Bombus$ sp.).

During nectar robbing, robbers did not contact the surface of the stigma, although they tended to vigorously shake branches. $D. albilatera$ pierced the corolla tube near the base with its lower mandible, while holding the flower with its upper mandible, leaving a highly visible slit in the tube. Usually, the robbery did not cause damage to the reproductive organs and none of the robbed flowers examined ($n = 250$) had a damaged style. However, when the robber forcefully shook the flower, this caused some release of pollen from the poricidal anthers, most of which fell to the ground but some adhered to stigmas within the pierced flowers. Our examination of flowers showed that 11.5% of them had been robbed at some point ($n = 1106$ flowers).

The number of visits varied over the day (Fig. 3). About half of all visits in the two patches occurred between 8:30 and 10:30 (mean 46.7 flowers per hour in the observed patches). Visit frequency then declined to 18.1 flowers per hour after 16:30. Visit frequency by all three main pollinators decreased towards the end of the day (Fig. 3).

### Table 1. Visitors to Disterigma stereophyllum in the study area. Number of floral visits for each of the two foraging modes and mean number (±SD) of flowers visited on each arrival.

<table>
<thead>
<tr>
<th>species</th>
<th>type of visit</th>
<th>no. of visit (%)</th>
<th>no. of flowers visited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trochilidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ocreatus underwoodii</td>
<td>P</td>
<td>2067 (49.5)</td>
<td>2.2 ± 3.7</td>
</tr>
<tr>
<td>Doryfera ludoviciae</td>
<td>P</td>
<td>70 (1.6)</td>
<td>11.7 ± 13.2</td>
</tr>
<tr>
<td>Colibri thalassinus</td>
<td>P</td>
<td>197 (4.7)</td>
<td>1.7 ± 2.0</td>
</tr>
<tr>
<td>Adelomyia melanogenys</td>
<td>P</td>
<td>45 (1.1)</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>Coeligena torquata</td>
<td>P</td>
<td>94 (2.4)</td>
<td>1.2 ± 0.6</td>
</tr>
<tr>
<td>Chlorostilbon mellisugus</td>
<td>P</td>
<td>1038 (24.8)</td>
<td>1.8 ± 2.1</td>
</tr>
<tr>
<td>Aglaiocercus coelestis</td>
<td>P</td>
<td>380 (9.1)</td>
<td>1.9 ± 3.6</td>
</tr>
<tr>
<td>Thraupidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diglossa albilatera</td>
<td>R</td>
<td>256 (6.1)</td>
<td>2.6 ± 1.9</td>
</tr>
<tr>
<td>Diptera</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diptera sp.</td>
<td>P</td>
<td>2 (0.05)</td>
<td>2.0 ± 0.0</td>
</tr>
<tr>
<td>Hymenoptera</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bombus sp.</td>
<td>P</td>
<td>29 (0.7)</td>
<td>14.5 ± 14.9</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>4178</td>
</tr>
</tbody>
</table>

$P =$ nectar extraction + pollination; $R =$ nectar robber making holes in corolla tubes. Numbers in brackets show the percentage of total visits.

![Fig. 3. Diurnal variation in relative frequency of visitors to flowers of Disterigma stereophyllum.](image)

**Fig. 4.** Number of pollen grains (mean ± SE) of Disterigma stereophyllum placed on the stigmas after a single visit by its main pollinators ($Ocr =$ Ocreatus underwoodii ($n = 11$); $Dor =$ Doryfera ludoviciae ($n = 8$); $Col =$ Colibri thalassinus ($n = 8$); $Chl =$ Chlorostilbon mellisugus ($n = 10$); $Agl =$ Aglaiocercus coelestis ($n = 9$); $Dig =$ Diglossa albilatera ($n = 8$). Bars with the same letter do not differ significantly (multiple comparisons test).
day, although only C. mellisugus showed zero activity during this last period.

Pollinator efficiency on pollen deposition

All pollen grains found on stigmas were conspecific (when the study was carried out, D. stereophyllum was the only species of this genus in flower, and its pollen is distinct from that of other ericads in the area). There were significant differences in the number of pollen grains found on the stigma after each visit ($F_{5,31} = 3.546$, $P = 0.0120$). Tukey tests showed that the differences were mainly due to the lower number of pollen grains found after D. albilatera visits (Fig. 4).

DISCUSSION

During our study, the flowers of D. stereophyllum were mainly visited by two territorial hummingbirds and a few ‘trapliner’ hummingbirds, with the two territorial species responsible for ca. 75% of visits. These pollinators show activity peaks early in the day, with activity subsequently declining. Diurnal patterns of this type have been observed in other tropical hummingbirds (Tiebout 1992), and have been attributed to the decline in nectar standing crop often observed towards the end of the day in hummingbird-pollinated flowers (Stiles 1975; Stiles & Wolf 1979). In the present study, we did not detect any significant decline in nectar availability towards the end of the day, but did observe a decline in nectar concentration. Castellanos et al. (2002) suggest that this floral mechanism could aid maintenance of pollinator visitation rates, providing a more constant reward (in terms of volume of nectar) to the pollinators. Regardless of this, the diurnal activity patterns observed here are very similar to those reported previously for other hummingbird-pollinated plants in the area, (Navarro 1999).

In spite of the lack of morphological fit with the flowers, all the species of hummingbird visiting D. stereophyllum show a good capacity to deposit pollen grains onto the stigma. Mayfield et al. (2001) showed that the ‘unexpected’ visitors for a syndrome can be as efficient during each visit, as the ‘right’ pollinator, or more so. Only D. albilatera, that behaves as a nectar robber (sensu Inouye 1983), showed reduced capacity to deposit pollen. In fact, this species did not contact the stigma during nectar robbing as some other nectar robbers do (Navarro 2000), but its shaking of the flower did cause some self-pollen to reach the stigma.

Disterigma stereophyllum apparently has a bee-pollination syndrome in its flower colour, size, shape and erect disposition. But it also shows certain floral characteristics that match those expected for the ornithophilous floral syndrome (Thomson et al. 2000) and that Luteyn & Sylva (1999) have suggested as adaptations to ornithophily in neotropical Ericaceae. These characteristics include style length equal to corolla length, rigid anthers arranged on the external face of the nectariferous disc, and abundant nectar with sucrose content of about 20% (Navarro et al. 2007). This dilute nectar places the species within the range classically reported for hummingbird-pollinated plants (Pyke & Waser 1981).

Luteyn (1989) suggests that the neotropical Ericaceae are switching from entomophily (the ancestral state) to ornithophily, with a superior ovary being replaced by an inferior ovary. In the genus Bejaria, generally considered to be one of the most primitive extant genera of the Ericaceae (Abbott 1936; Camp 1941; Copeland 1943), B. aestuans for example shows white or pink corollas with distinct extended petals, and is normally insect-pollinated, while B. resinosa has imbricate petals that produce a red tubular corolla and is hummingbird pollinated (Melampy 1987; Luteyn 1989; see however Kraemer 2001). Similar evolution of floral characteristics has been reported in other families with neotropical members, such as the Scrophulariaceae (Kampny 1995). Studies on Disterigma have indicated that most species of this genus produce small quantities of highly concentrated nectar and are visited by bees (Snow & Snow 1980; Luteyn & Sylva 1999; but see Bleiweiss & Olalla 1983). As pointed out above, this is not the case for D. stereophyllum, which produces large amounts of relatively dilute nectar, in accordance with a strong predominance of hummingbird visits in our study area (Table 1). In fact, D. stereophyllum shows what has been called a ‘mixed pollination system’, a combination of floral characters that are consistent with pollination by both insects and birds (Navarro et al. 2007). Similar patterns have been reported for Alepis flavida, a Loranthaceae from New Zealand (Ladley et al. 1997) and for Penstemon pseudoespectabilis (Scrophulariaceae), which attracts frequent hummingbird visits, but which retains the capacity for self-pollination and may be also pollinated by bees (Lange & Scott 1999). Wilson et al. (2006) suggest that ‘despecialised’ stages might occur in the transition from the bee to the hummingbird pollination syndrome in Penstemon. But, in our case, unfortunately, it is still not possible to infer any evolutionary pathway because the phylogeny of the genus is still incomplete (but see Powell & Kron 2003; Pedraza 2006).

In conclusion, the results of this study suggest that D. stereophyllum is basically pollinated by hummingbirds, despite the fact that its floral morphology does not fully match the hummingbird floral syndrome. We should stress, however, that pollinators of the species could vary through its geographic range, and could vary through time; it is always somewhat risky to draw overall conclusions from a single study. But taking our results at face value, D. stereophyllum seems to be another of the growing list of species for which there is no clear relationship between the usual pollinator spectrum and floral traits. More detailed elucidation of this question will require further ecological studies of this and related species. To this end, also, more studies are necessary to resolve the group phylogeny and to evaluate if there has been an evolution toward hummingbird pollination from entomophilous ancestors, as suggested by Luteyn (1989) for neotropical Ericaceae and as shown in other neotropical genera (e.g. Costus, Kay et al. 2005).
ACKNOWLEDGEMENTS

We thank the personnel of the Reserva Natural La Planada (Nariño, Colombia) who pulled out all the stops to make it possible for us to work in this beautiful location. The comments of Marcos Mendez and Jim Luteyn on a preliminary version substantially improved this manuscript. We are very grateful to Nick Waser and to an anonymous reviewer who made very constructive revisions. We would also like to thank Jens Bittner for help monitoring fruit set levels. The work was partially financed under grants PGIDT04P-XIC31003PN from the Spanish Ministerio de Ciencia y Tecnología and the FEDER funds from the European Union to L.N. and a travel grant from the University of Santiago to P.G.

REFERENCES


