

Adaptation of floral traits and mating system to pollinator unpredictability: the case of *Disterigma stereophyllum* (Ericaceae) in southwestern Colombia*

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Abstract. The Neotropical group of Ericaceae offers a variety of floral displays. Here we present data on floral characteristics and breeding system of *Disterigma stereophyllum*, a Neotropical epiphyte Ericaceae abundant in semi-disturbed areas with pollinator unpredictability. Floral characteristics are intermediate between those typical of bee- and hummingbird-pollinated plants: the corolla is short, white, and urceolate, but the composition and amount of nectar are similar to those typically observed in hummingbird-pollinated species. Plants are capable of autonomously self-pollination, but manual outcrossing yielded more than twice as many fruits as self hand-pollination did. Moreover, outcross pollen grows more rapidly than self pollen down the style. Our results provide another example of a species with a “mixed pollination syndrome” which is cryptically self-incompatible and preferably attracts high-energy pollinators (hummingbirds), but which can also profit from small bee visitors and retains the ability to self-pollinate just in case. We hypothesize that interaction among several factors, including self pollinating possibility, and the capacity to attract a wide pollinator spectrum, may result in a lack of strong selection

for a bee vs. a hummingbird floral morphology, thus allowing the persistence of mixed floral traits in these ericads. The maintenance of these traits may enhance the ability of this species to colonize semi-disturbed areas.

Key words: Colombian Andes, Ericaceae, floral morphology, pollination syndrome, pollination limitation, plant breeding system.

Animal-pollinated plants govern their mating opportunities through the effect of floral design and reward on pollinator attraction and pollen dispersal. The floral traits that characterize floral design and rewards can thus modify the actions of pollen vectors so as to enhance reproductive success. If floral characters affect pollination, and pollination affects mating, then the functional link between pollination and mating implies that selection that increases reproductive success will often result in correlated evolution of floral characters and mating systems. In fact, some associations between floral characters, pollination systems, and mating systems have been reported (Ornduff 1969; Wyatt 1983; Diggle

* This paper is devoted to the memory of Martha Janeth Portillo.

1992; Armbruster 1993, 1997; Brunet and Eckert 1998; Ashman 2000; Lange et al. 2000), and also, there is some evidence of actual influence of pollinators on floral characteristics and mating systems on some plant species (Campbell 1991, 1996; Campbell et al. 1996; Meléndez-Ackerman et al. 1997; Schemske and Bradshaw 1999).

However, this evidence has been found in systems without wide variations on the pollinator spectrum. But actually many interactions between plants and pollinators occur in semi-disturbed habitats (Kearns et al. 1998). Several authors have shown that in disturbed and partially-disturbed areas, the number and spectra of pollinator visits to a plant species would be more unpredictable (Aizen and Feinsinger 1994a, b; Kearns et al. 1998). Moreover, Fenster et al. (2004) suggest that when a plant is visited by several functional groups, the relative selective pressures they exert are likely to differ greatly. In this sense, it would seem be of increasing interest to focus empirical effort on analyzing the floral characteristics of plant species that inhabit semi-disturbed areas, in search of mixed pollination systems. These systems combine floral traits inherent to more than one pollination syndrome.

The Ericaceae are a cosmopolitan family of some 110 genera and more than 4000 species, with greatest diversity in the montane Neotropics. The blueberry subfamily (Vaccinioideae), with over 1000 species, is especially abundant in the tropics of the New World, Malaysia, and southeastern Asia. There they usually occur as epiphytic shrubs in the cool, moist montane regions referred to as cloud forests, although a few species range into typically tropical situations. In the Neotropics, Ericaceae are concentrated in northwestern South America, in the cool, moist, montane forest habitats between 1200 to 3000 m elevation, primarily in Colombia, Ecuador, Peru, and Venezuela, where nearly 50% of the species are epiphytes and approximately 94% are endemic (Luteyn 1989). Flowers of most Neotropical montane Ericaceae species are

showy, and there is much variation among species, suggesting that adaptation to a variety of pollinators has been important in the adaptive radiation of the family. However, in spite of the interesting characteristics of this family, published information on floral biology and/or pollination ecology is still unavailable for most Neotropical species (see however Melampy 1987; Murray et al. 1987; Murcia and Feinsinger 1996; Luteyn and Silva 1999; Navarro 1999, 2001; Busby 2000; Kraemer 2001). Luteyn (1989) states that pollination of Ericaceae in temperate and subtropical latitudes is primarily carried out by bees, but in the Neotropics bird-pollination is the rule, with hummingbirds acting as the main vector. However, within Neotropical ericads there are many floral morphologies and syndromes. For this reason we decided to examine the relationship between floral characters, mating system and pollinator specificity on this diverse family. In previous work in the Colombian Andes we have examined the reproductive biology of *Macleania bullata* (Navarro 1999, 2001), a Neotropical Ericaceae whose flowers show a typical hummingbird-pollination syndrome. This species has a xenogamous mating system and presents specialized floral characteristics corresponding to long-billed hummingbird pollination. In the present paper, we analyze the relationships between the floral morphology and mating system in a short urceolate flowering plant inhabiting semi-disturbed areas.

The genus *Disterigma* is one of the largest genera of Neotropical ericads. It includes 40 species ranging approximately from Guatemala south to Bolivia and east to Guyana (Luteyn 1989). Here, we focus on the reproductive biology of *Disterigma stereophyllum*, a Neotropical Ericaceae. Our aim was to investigate possible associations between the floral traits (including morphology, nectar production, P:O ratio and stigma receptivity) and mating system in this species that is found in semi-disturbed areas where pollinator limitation could take place (Navarro 2001). This work forms part of a broader study of the

reproductive biology and pollination ecology of the Ericaceae of Neotropical montane forests.

Materials and methods

The study was carried out in the Reserva Natural La Planada, near the village of Ricaurte (Departamento de Nariño, Colombia) (1°10' N; 77°58' W) during 1998. The reserve is located on the western flank of the Andes, and comprises 3200 ha of montane wet forest (bmh-PM sensu Holdridge 1996) at altitudes of between 1200 and 2100 m. Mean annual precipitation is 4375 mm, and annual mean temperature ranges between 12°C and 23°C. More information about the study area is given in Orejuela (1987) and Restrepo and Gómez (1998).

Disterigma stereophyllum (A. C. Smith) Luteyn (Ericaceae) is an epiphyte which, in the study area, typically forms part of the semi-disturbed fringe community around mature forest. It has several flowering peaks during the year. Flowers are hermaphroditic with a fleshy, white, tubular corolla with purple lobules (Fig. 1a). The style is slightly exerted and overtops the short anthers (Fig. 1c). Flowers are nectar-producers and except for the pollen this species did not present any other floral reward. In 1998 pollinator censuses carried out during 66 hours of March-April on two floral patches located in the same area, showed that, for a total of 4178 recorded visits, two trapliner hummingbirds were responsible for about the 75% of visits, whereas bumblebees made up only the 1% of the visits. The main flower visitor in the study area was the hummingbird *Ocreatus underwoodii*, which accounted for 50% of visits, followed by *Chlorostilbon mellisugus*, which accounted for 25% of visits. Flowers also received visits from nectar robbers, but the frequency of robbed flowers in the study population was low (11.5%) (Navarro et al., unpublished data).

The fruit, a translucent white berry with more than 100 seeds (Fig. 1b), is eaten by frugivorous birds (*Tangara arthus* and *Euphonia xanthogaster*) (Navarro, unpublished data). Voucher specimens of *Disterigma stereophyllum* from the study site are deposited at the herbarium of the Universidad de Pasto (Departamento de Nariño, Colombia).

Floral morphology. In 14 flowers (one per plant), we measured corolla length, corolla width at the opening of the tube, style length and stamen

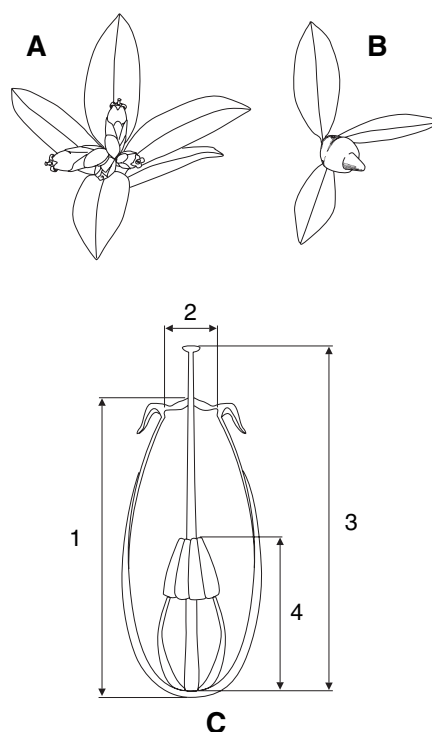


Fig. 1. Aspects of *Disterigma stereophyllum* flowers (A), fruit (B) and morphometric variables studied on flower (C): 1 corolla length; 2 corolla width at the opening of the tube; 3 pistil length; 4 stamen length

length (see Fig. 1c). In another nine flowers we counted the number of ovules and pollen grains per flower. For this, flowers were cut just before anthesis, and ovules were counted under a dissecting microscope. Number of pollen grains was estimated by counting in a Newbauer chamber following Dafni (1992).

Floral duration. To investigate floral duration we marked a total of 22 flowers (one per plant) before they opened, and monitored them daily.

Stigma receptivity. To investigate stigma receptivity, we used peroxidase test paper (Peroxtesmo Ko, Macherey-Nagel, Co., Carle Place, New York; cat. no. 90606) in flowers aged from 0 to 6 days (one flower per plant, 12 flowers per age class). If the stigma is receptive, as indicated by the presence of peroxidase, the test paper changes colour.

Nectar production. To investigate nectar production over 24 hours, we bagged 25 randomly chosen flowers on 10 plants and, after 24 h, we determined nectar volume in each flower with a capillary micropipette. We also determined sugar

concentration on site, with a portable refractometer (Fisher, mod. 0–32%). On the basis of these data we estimated (following Cruden and Hermann 1983) the amount of sugar produced by each flower in 24 h. Nectar was extracted after removing the corolla to avoid contact between the micropipette and anthers or stigma.

Effects of pollen source on fruit set. To investigate the importance of pollinators on pollen transfer, and to examine the effect of pollen source on fruit set levels, we tagged 10 randomly selected branches on 10 individual plants. On each branch a group of flowers was tagged and not manipulated, as the population control ($n = 101$ flowers from 10 plants), while another group was pollinator-exposed but also cross-pollinated by hand daily (supplementary pollination; $n = 96$ flowers, from 10 plants). Other groups of flowers were treated as follows:

Pollinator exclusion: bagged with mosquito netting ($n = 66$ flowers, from 10 plants).

Autogamy: bagging then hand-pollination every day after anthesis with pollen from the same flower ($n = 45$ flowers, from 10 plants).

Xenogamy: bagged flowers carefully emasculated and hand-pollinated every day after anthesis with pollen from another plant ($n = 44$ flowers from, 10 plants).

Fruit set was recorded after approximately 2–3 months. Two indices related to selfing were calculated for fruit set, following Lloyd and Schoen (1992): 1) the self-fertility index, calculated as [fruit set after spontaneous selfing] / [fruit set after hand outcrossing, i.e. xenogamy], which estimates the ability to set fruit in the absence of pollinators, and 2) the self-compatibility index, calculated as [fruit set after hand selfing, i.e. autogamy] / [fruit set after hand outcrossing, i.e. xenogamy].

Effects of pollen source on pollen tube growth. To examine whether or not there is any physiological barrier to penetration of the style by self pollen we compared the rate of pollen tube growth following spontaneous self-pollination (untreated bagged flowers), hand self-pollination and hand cross-pollination. Batches of 19–27 flowers were used per treatment. In addition, 20 unmanipulated flowers were randomly selected as the population control. Styles were collected and fixed in FAA 6 h after pollination, then preserved in 70% ethanol. In the laboratory, styles were softened in 8 M NaOH overnight, and stained

with 0.1% aniline blue in 0.1 M K_3PO_4 (Martin 1959). Squash preparations were examined with an epifluorescence microscope. We counted the number of pollen tubes at three intervals along the style (top, middle, bottom) expressed as percentages of the number of pollen grains deposited on each stigma.

In basis of pollen transfer experiments in fruit set and pollen tube growth we infer the breeding systems of this species.

Data analysis

To investigate whether flower morphometric variables covary, a principal component analysis was performed. Traits values were natural log-transformed prior to statistical analysis to eliminate heterocedasticity.

Fruit sets were compared among the pollen transfer treatments and the population control using two-way ANOVA for a randomized block design, with plant as blocking factor. Subsequent pairwise comparisons were by Tukey tests. The results of the pollen-tube-growth experiments were analyzed by one-way ANOVA (factor pollen transfer treatment), again with Tukey tests for subsequent pairwise comparisons. All percentage data were subjected to an arcsine transformation before analysis of variance. All analyses were carried out with the statistical package SYSTAT (Systat 1997). In the text, mean values are cited with their standard errors.

Results

Floral morphology. Flowers of *Disterigma stereophyllum* have an erect urceolate corolla (Fig. 1c). The mean length of the corolla tube was 8.8 ± 0.2 mm (coefficient of variance = 7.5%), and the style slightly overtops the corolla rim (mean style length 9.0 ± 0.2 mm; coefficient of variance = 9.1%). The anthers surround the style, and are about 1 mm shorter than the style (mean anther length 7.9 ± 0.2 mm; coefficient of variance = 7.2%) generating a slight approach towards herkogamy. Nectar is secreted at the bottom of the corolla.

The mean diameter of the corolla tube at the apex is 1.0 ± 0.1 mm (coefficient of variance = 20.7%). The mean number of ovules per flower is 362 ± 30 (range = 252-501). The mean number of pollen grains per flower is 31875 ± 5360 (range = 8125-55000). The mean P:O ratio was 92 ± 17 ($n = 9$ flowers).

When we subjected floral traits to a principal components analysis we observed that the first axis extracted explained 65.8% of total variance and the second 23.3%

Table 1. Summarized results of a principal components analysis of the floral variables matrix in *Disterigma stereophyllum*, showing loadings of each variable on the first two axes extracted

Variable	Axis	
	I	II
Corolla length	0.977	0.0007
Stigma length	0.915	0.142
Anthers length	0.826	0.283
Corolla diameter	0.398	0.912
%variance explained	65.78	23.29

(Table 1). Corolla length, style length and stamens length had high loadings on the first factor, indicating that these floral traits tend to covary. The variation on the corolla width at the opening of the tube is explained by the second axis.

Floral biology. Mean floral lifespan was 4 ± 1 days ($n = 22$ flowers). Flowers are protandrous. Anther dehiscence occurred when the flowers opened, and the stigma was not completely receptive until the third day (Fig. 2). Nectar was located exclusively at the bottom of the corolla tube. The mean volume of nectar produced by a flower in 24 hours was 48 ± 49 μ l, and its mean sugar concentration was $21.2 \pm 6.6\%$ weight/weight.

Effects of pollen source on fruit set. The mean population-control fruit set was $28.0 \pm 9.1\%$. The mean fruit sets of both xenogamous ($59.7 \pm 6.4\%$) and pollen supplemented treatments ($40.5 \pm 6.7\%$) were significantly higher than the mean fruit sets of both autogamous treatment ($23.0 \pm 7.0\%$) and bagged untreated flowers ($31.4\% \pm 13.9\%$) ($F_{4,36} = 3.6$, $p = 0.0142$). Furthermore, there was no significant variation among population

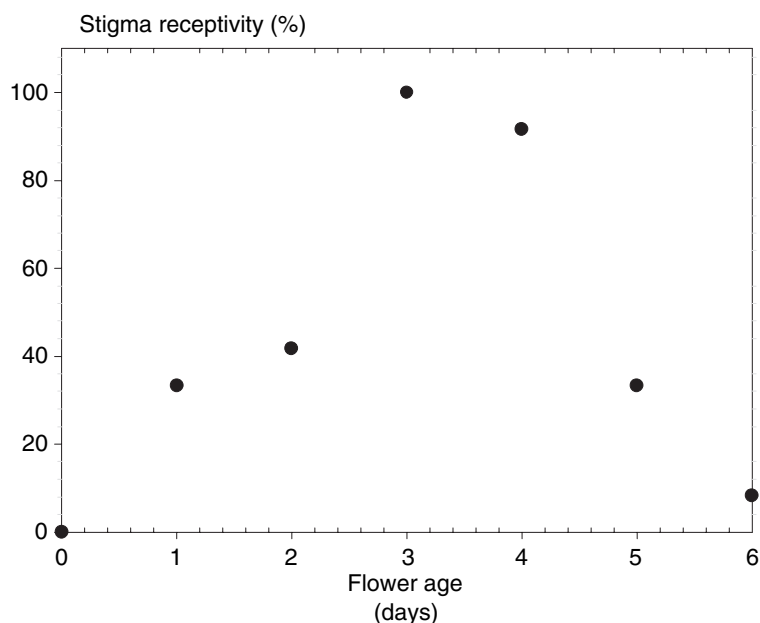


Fig. 2. Variation in stigma receptivity with flower age in *Disterigma stereophyllum*. The vertical axis shows the proportion of flowers with receptive stigma on that day

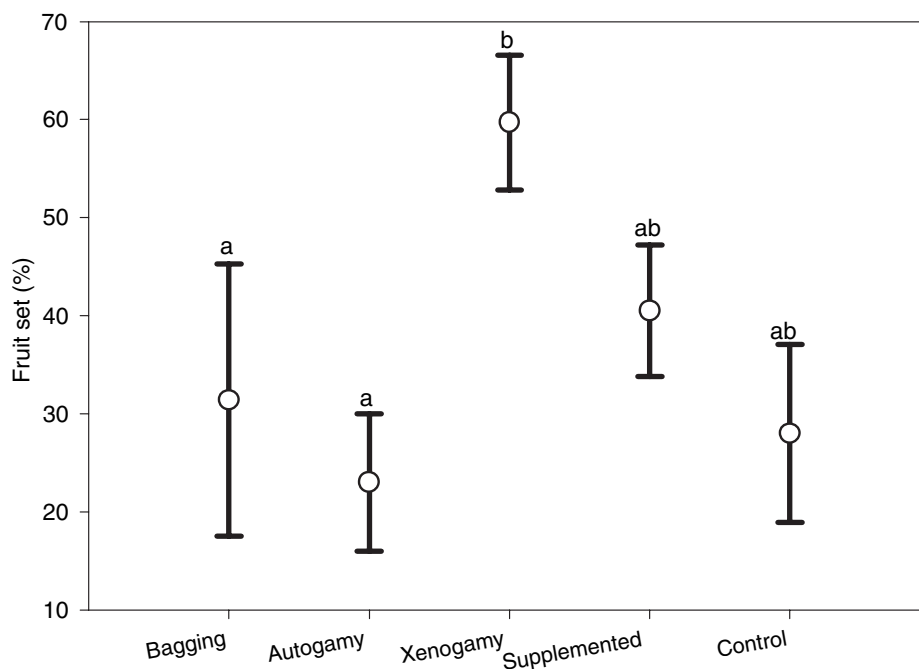


Fig. 3. Mean (\pm SEM) fruit set (%) by *Disterigma stereophyllum* under natural conditions (control) and following different pollen transfer treatments: bagging (i.e. spontaneous selfing), autogamy (i.e. bagging and brush pollination with same-flower pollen), xenogamy (i.e. bagging, emasculation and brush pollination with outcross pollen) and supplemented (i.e. exposure to pollinators, and brush pollination with outcross pollen). Means with the same letter do not differ significantly at the 5% level (Tukey tests)

controls and flowers subjected to either of the autogamy treatments (Fig. 3). The factor plant was not a significant source of variation ($F_{9,36} = 1.7$, $p = 0.1140$). The self-fertility index was of intermediate magnitude (0.53), whereas the self-compatibility index was low (0.39).

Effects of pollen source on pollen tube growth. Pollen-transfer treatment had clear effects on pollen tube growth down the stigma (Fig. 4). Neither the number of pollen grains deposited on the stigma nor the proportion of pollen grains germinating varied significantly among treatments (number deposited: $F_{3,86} = 1.1$, $p = 0.3511$; proportion germinating: $F_{3,85} = 2.4$, $p = 0.0711$). In all treatments, only a relatively small proportion of pollen tubes penetrated beyond the top sector. Attrition between the top sector and the middle sector was however less severe in the case of outcross pollen, as reflected by the significant among-treatment variation in the proportion

of tubes reaching the middle sector ($F_{3,85} = 7.8$, $p = 0.0001$). Among-treatment variation in the proportion of tubes reaching the bottom sector was more pronounced ($F_{3,85} = 22.8$, $p < 0.0001$), reflecting the marked difference between the proportions of self pollen and outcross pollen reaching this sector. Furthermore, the proportion of pollen reaching the bottom sector was markedly higher in the population control than in the autogamy treatment (Tukey tests, $p < 0.05$), presumably reflecting the presence of outcross grains in the population-control flowers.

Discussion

The Neotropical group of Ericaceae offers a variety of floral displays in which two common syndromes can be observed: widely urceolate white-purple flowers, apparently bee-adapted, and narrower, tubular, red flowers, apparently

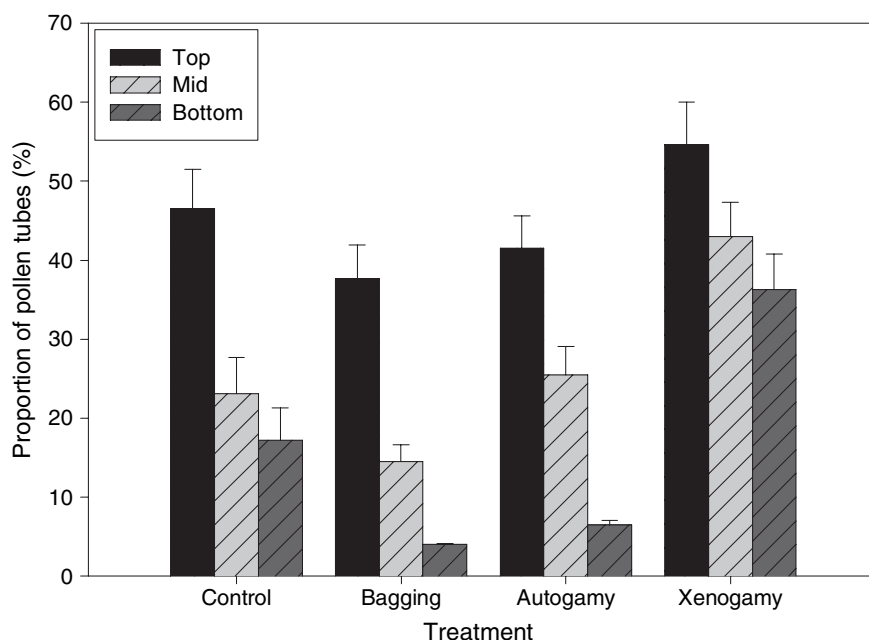


Fig. 4. Mean proportion (\pm s. e.) of pollen tubes reaching different positions down the style, for each pollen transfer treatment. The pollen transfer treatments are as defined for Fig. 3 (pollen supplementation excluded)

hummingbird-adapted flowers. For a plant species colonizing semi-disturbed areas where both pollen limitation and pollinator unpredictability may take place (Aizen and Feinsinger 1994a, b; Kearns et al. 1998), a set of floral traits attracting the greatest possible number of pollinators will improve pollen receipt and removal. Moreover, if the species shows cryptic self-incompatibility (Bateman 1956), the probability of a successful production of fruits and seeds is greatly enhanced.

The results of the present study show that *Disterigma stereophyllum* does not present just one floral syndrome, rather it combines floral characteristics typical of ornithophilous and melittophilous species (see Table 2). According to what was reported by Luteyn and Silva (1999), like typical adaptations of Neotropical Ericaceae to ornithophily, *Disterigma stereophyllum* presents a) style length equal to the corolla length, b) rigid anthers attached to the outside of a nectariferous disk and forming a tunnel leading to the nectar source, and c) abundant nectar with a sucrose content of about 20%. However, erect flower disposition, the lack of flexible pedicels, small flower size,

urceolate shape and white colour in *Disterigma stereophyllum* are all typical floral traits of melittophilous species (see Thomson et al. 2000 for a recent revision on floral syndromes). On the other hand, the corolla length, style length, and anther length tend to covary, pointing to the existence of an “integrated floral phenotype”, which characterizes plants with a specialized pollination system

Table 2. Relationship of floral characters studied on *Disterigma stereophyllum* with hummingbird- or bee-pollinated plant species (following Luteyn and Silva 1999, Thomson et al. 2000)

Floral characters	Hummingbird	Bee
Corolla small, white and urceolate	No	Yes
Abundant nectar of low sugar concentration	Yes	No
Style length equal to corolla length	Yes	No
Rigid anthers forming a tunnel	Yes	No
Corolla erect	No	Yes
Lack of flexible pedicels	No	Yes

maintained by constant specialized visitors (Berg 1960, Armbruster et al. 1999). However, the corolla width shows very little correlation with the rest of the floral traits measured. This lack of covariation between the corolla width and the rest of correlated traits could have, at the very least, one possible explanation: the corolla width in a plant species that maintains a mixed-pollination syndrome could not be subjected to selective pressure in order to permit access to the interior of the corolla to a broad array of pollinators (i.e. hummingbirds or bees) (see Berg 1960).

Moreover, the pollen-transfer experiments that we carried out would indicate that *D. stereophyllum* is cryptically self-incompatible (Bateman 1956). Fruit set after outcross pollination is more than twice as high as after selfing. Furthermore, our results indicate that outcross pollen shows better penetration of the style than self pollen. Some authors have suggested that the balance between cross- and self-pollination is typically a function of pollinator activity, which may be negatively affected by cold and/or rainy conditions (Cruden and Lloyd 1995). Bearing in mind that *D. stereophyllum* is abundant in woodland-fringe communities and semi-disturbed areas, where pollinator availability might be more unpredictable (Aizen and Feinsinger 1994 a, b; Kearns et al. 1998), a cryptic self-incompatibility system would facilitate fruit and seed production when pollinators are scarce. In fact, the results of our pollen-transfer experiments showed that the fruit set of population control plants in the year of study was similar to that obtained after selfing, which would suggest pollen limitation.

In the Neotropics, Ericaceae predominantly occur in moist, semi-disturbed habitats in cool montane regions (Luteyn 1989). Luteyn (1989) suggests that the strong colonization capacity of many of these species means that they must be self-compatible. In previous studies carried out in the same area, Navarro (1999, 2001) studied the pollination and breeding ecology of *Macleania bullata*, a long-billed-hummingbird-pollinated flower,

which has typical floral traits of hummingbird-pollinated flowers (red long-tubular and herkogamous flowers) and presents self-incompatibility. In such species there may be a strong selective pressure that favored some pollinator specialization in floral traits (see Schemske and Bradshaw 1999). Our results on *D. stereophyllum* indicate that this species does not suffer a strong selection for a bee versus a hummingbird floral morph. This allows for the maintenance of mixed floral traits which is advantageous to these unpredictable habitats. The “mixed” set of morphological floral traits in this species favors pollen transfer by any of a wide range of pollinator species in the area. Moreover, *D. stereophyllum* retains the capacity for selfing, which in case of pollinator scarcity or poor pollinator efficiency, may serve to assure reproduction (Eckert and Schaefer 1998). Similar cases have been described for *Alepis flavida* (Loranthaceae) and for *Penstemon pseudospectabilis* (Scrophulariaceae) which attract frequent hummingbird visits, but which retain the capacity for self-pollination and may be pollinated by bees (Ladley et al. 1997, Lange and Scott 1999). The Neotropical Ericaceae *Bejaria resinosa* also shows red tubular corollas and is pollinated mainly by hummingbirds in low areas (Melampy 1987, Luteyn 1989) and by bumblebees at 3700 m altitude (Kraemer 2001). This author points to the shortage of long-billed hummingbirds in the area of study and to the ability of this species to attract other pollinators.

This type of “pollination syndrome” flexibility combined with a cryptic self-incompatibility certainly enhances these species’ capacity to colonize disturbed areas. More detailed future studies of the reproductive systems and floral characteristics of the different Neotropical species of this family would help us assess the extent to which these traits are responsible for their success in montane habitats. Although some authors (see Ollerton 1998) have concluded that generalization is the rule rather than the exception for angiosperms at a global level, the truth is that little is known

about the pollination systems of the vast majority of plants in the species-rich countries of the world (Johnson and Steiner 2000). In this sense, this study contributes to the knowledge of pollination syndromes in both species-rich areas such as the Colombian Andes and in plant families such as the Ericaceae that are rich in floral forms.

This paper is devoted to the memory of Martha Janeth Portillo, who was killed by an armed group when working at the Reserva Natural La Planada. 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 at this beautiful but, unfortunately, dangerous location. We also thank Jens Bittner for helping monitoring fruit set levels, Guy Norman and Miguel Salvande for their useful suggestions on the manuscript, and Luis Gómez Orellana, who drew Fig. 1. Claire Teed reviewed the final version improving the English. The comments and suggestions from two anonymous reviewers substantially improved the final version of this manuscript. The work was supported in part by a grant from the University of Santiago to P.G. Manuscript preparation was also partially financed under grants PGIDT04PXIC31003PN from the Xunta de Galicia and BOS2003-07924-CO2-02 from the Spanish DGICYT to L.N.

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