

RESEARCH PAPER

Reproductive biology and pollination mechanisms of *Epidendrum secundum* (Orchidaceae). Floral variation: a consequence of natural hybridization?

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ABSTRACT

The phenology, flower morphology, pollination mechanism and reproductive biology of *Epidendrum secundum* were studied in a semi-deciduous forest at the Serra do Japi (SJ), and in the Atlantic rain forest of Picinguaba, both natural reserves in the State of São Paulo, southeastern Brazil. *E. secundum* flowers all year round, with a flowering peak between September and January. This species is either a lithophytic or terrestrial herb in the SJ, whereas, in Picinguaba, it grows mainly in disturbed areas along roadsides. *E. secundum* is pollinated by several species of diurnal Lepidoptera at both study sites. In Picinguaba, where *E. secundum* is sympatric with *E. fulgens* and both share the same pollinators, pollen transference between these two species was recorded. *E. secundum* is self-compatible but pollinator-dependent. It is inter-compatible with *E. fulgens*, producing fertile seeds. In contrast to the population of the SJ, in the Picinguaba region, floral morphology is quite variable among plants and some individuals present flowers with characteristics in-between both sympatric species, suggesting that natural hybridization occasionally occurs. The anthropogenic perturbation is probably the cause of the occurrence of *E. secundum* in the Picinguaba region, enabling its contact with *E. fulgens*.

INTRODUCTION

Epidendrum L. (tribe Epidendreae, subtribe Laeliinae) is a large genus belonging to the orchid family. It has almost 1000 native species distributed throughout tropical America, from Florida to northern Argentina (Dressler 1993; Morrison 1997). As in most of the South American species that are often misnamed *Epidendrum ibaguense* Lindl., *Epidendrum secundum* Jacq. is a member of the 'Epidendrum secundum complex' (Dressler 1989). The numerous forms belonging to the 'E. secundum complex' are common along roadsides at high elevations in Central and South America and in the West Indies (Dressler 1989), and occur in disturbed areas of Venezuela (Dunsterville 1979).

According to Dodson & Frymire (1961), *E. secundum* is pollinated by *Urbanus proteus* L. (Hesperiidae). Dodson (1962a) reported skippers as co-pollinators of *E. secundum*, and mentioned having observed

hummingbirds of the genus *Amazilia* as further pollinators of this orchid species. According to Van der Pijl & Dodson (1966), an unidentified skipper and *Papilio polyxenes* Fabricius (Papilionidae) are pollinators of *E. secundum* in Costa Rica. Some *Epidendrum* species closely related to the 'E. secundum complex', such as *E. ibaguense* and *E. radicans* Pavón ex Lindl., do not produce nectar and mimicry species of *Lantana* and *Asclepias* are thus pollinated by deceit (Boyden 1980). Food-deception is a common pollination system in many Orchidaceae (e.g. Ackerman 1986; Montalvo & Ackerman 1987; Ackerman & Montalvo 1990; Calvo 1990; Christensen 1992; Johnson 2000), which are estimated to represent at least one-third of the orchid family (Ackerman 1986). Orchid species pollinated by deceit have low-reproductive success under natural conditions, mainly because of the low frequency of effective pollinators on their flowers (e.g. Montalvo & Ackerman 1987; Ackerman 1989; Zimmerman & Aide 1989).

Pre-pollination barriers are the main factor to prevent hybridization in Orchidaceae (Dodson 1962a,b; Van der Pijl & Dodson 1966; Dressler 1981). When these barriers are broken or do not exist, however, hybrids occasionally occur (e.g. Dodson 1962a,b; Van der Pijl & Dodson 1966; Romero & Carnevalli 1990, 1991a,b, 1992; Borba & Semir 1998; Levin 2000). In southern Ecuador, two colour forms of *E. secundum* grow side-by-side on rock faces and seldom is an intermediate form found. It appears that each form is pollinated by a different species of hummingbird, each of them attracted by a different colour. In northern Ecuador a bewildering hybrid swarm occurs, with the two colour forms of *E. secundum* as parents. The whole complex seems to be pollinated and sustained by a third hummingbird species, but does not occur in southern Ecuador (see Van der Pijl & Dodson 1966). Hybridization between different forms of *E. secundum* and other species belonging to the '*E. secundum* complex' was also documented by Dressler (1989). One of these hybrids is *Epidendrum xobrienianum*, an artificial cross between *E. radicans* and *E. evectum* Hook. According to Dressler (1989), this hybrid possibly 'escapes' from gardens, being sub-spontaneous in areas as far apart as Mexico and Africa. The introduction of these specimens in herbaria causes taxonomic difficulties to taxonomists studying them (Van der Pijl & Dodson 1966; Dressler 1989).

The present study reports the pollination mechanism, reproductive biology and fruit set of *E. secundum* under natural conditions. Although information about pollinator species (Van der Pijl & Dodson 1966) and hybridization among species belonging to the '*E. secundum* complex' (Dressler 1989) are present in the literature, they are fragmentary. Furthermore, this work provides the first report on the breeding system and reproductive success of a species belonging to the '*E. secundum* complex'. Based on flower variation and on evidence of pollen transference between plants of *E. secundum* and another related species (*E. fulgens* Brongn.), which occur sympatrically in the Picinguaba region, the possible occurrence of natural hybridization is discussed.

MATERIALS AND METHODS

Study sites

The pollination mechanisms and reproductive biology of *E. secundum* were studied in a mesophytic semi-deciduous forest in the Serra do Japi (SJ), district of Jundiá (approx. 23°11' S, 46°52' W; 700–1300 m.a.s.l.), and in the Atlantic rain forest of Picinguaba (approx. 23°33' S, 45°04' W; 0–50 m.a.s.l.), district of Ubatuba. Both areas are natural reserves of the State of São Paulo, Brazil. The SJ is located inland and Picinguaba is on the coast. The SJ is about 350 km from Picinguaba. In the SJ, annual rainfall is about 1500 mm, and annual average temperature circa 17.5 °C (Pinto 1992). This region is mainly characterized by semi-deciduous mesophytic forests of medium altitude with occasional rocky outcrops

(Leitão-Filho 1992). In Picinguaba, the annual rainfall is about 2624 mm, and the average temperature circa 21 °C (Nimer 1977). This region is characterized by evergreen Atlantic rain forests on steep slopes and 'restinga' vegetation covering the coastal plain on sandy saline soils.

Phenology and floral features

The phenological data on *E. secundum* were gathered by visiting both study areas each month, from March 1998 to February 2000. During these visits, the production of flowers, their duration and fruit dehiscence were recorded. Fresh flowers of *E. secundum* collected at both study sites, as well as flowers of *E. fulgens* and putative hybrids collected in the Picinguaba region, were studied under a binocular stereomicroscope and photographed. The measurements were made directly from floral structures using a caliper rule. To understand the variation in floral characters, as many flowers taken from different plants as possible were examined. Plant vouchers were deposited in the herbarium UEC at the Universidade Estadual de Campinas. Voucher numbers and location are presented in Table 1.

For anatomical study, fresh flowers of *E. secundum* collected at the SJ were fixed in 50% FAA. The labella were dehydrated in an ethanol series and embedded in glycol methacrylate. Cross sections were obtained using a microtome. Sections 9–12-µm thick were stained with toluidin blue (Sakai 1973).

Pollinators and pollination mechanisms

Field visits were undertaken at both sites to observe and record the pollination process and visitation frequencies, and to capture pollinators of *Epidendrum secundum* for

Table 1. *Epidendrum* species and putative hybrids, with their respective location and voucher numbers.

species	voucher	location
Putative hybrid 1	<i>E.R. Pansarin 528</i>	Ubatuba
Putative hybrid 2	<i>E.R. Pansarin & L. Mickeliunas 1203</i>	Ubatuba
<i>Epidendrum secundum</i> Jacq.	<i>E.R. Pansarin 530</i>	Ubatuba
<i>E. secundum</i> Jacq.	<i>E.R. Pansarin 190</i>	Serra do Japi
Putative hybrid 3	<i>E.R. Pansarin 529</i>	Ubatuba
Putative hybrid 4	<i>E.R. Pansarin 713</i>	Ubatuba
Putative hybrid 5	<i>E.R. Pansarin 531</i>	Ubatuba
Putative hybrid 6	<i>E.R. Pansarin 210</i>	Ubatuba
Putative hybrid 7	<i>E.R. Pansarin & L. Mickeliunas 1207</i>	Ubatuba
Putative hybrid 8	<i>E.R. Pansarin & L. Mickeliunas 1205</i>	Ubatuba
Putative hybrid 9	<i>E.R. Pansarin & L. Mickeliunas 1206</i>	Ubatuba
<i>Epidendrum fulgens</i> Brongn.	<i>E.R. Pansarin 712</i>	Ubatuba

later identification. In the SJ, the visits occurred from 20 March to 17 April 1998, 29 July to 23 November 1998, and from 2 to 4 December 1999, a total of 43 h of observation. In the Picinguaba region, visits took place from 21 to 26 July 1998, 23 to 25 April 1999, and 9 to 13 November 1999, a total of 37 h. In the Picinguaba region, additional observations were made on plants of *Epidendrum fulgens* occurring among populations of *E. secundum*. Observations were performed only on sunny days because we observed that cloud cover and precipitation affect butterfly visitation on flowers. In both study sites, the daily period of observation was from 7:30 to 17:30 h. At the end of each day of observation, unpollinated flowers were tagged and examined in the early morning, at about 7:30 h, to detect possible nocturnal pollination.

The insects collected while visiting the flowers were identified and vouchers were deposited at the Natural History Museum of the Universidade Estadual de Campinas (ZUEC). The Lepidoptera specimens were identified using the survey work performed by Brown (1992) in the SJ region.

Breeding system and natural fruit set

The breeding system of *Epidendrum secundum* was investigated using plants collected at both study areas, which were maintained in the greenhouse of the Universidade Estadual de Campinas, Campinas, State of São Paulo (22°49' S, 47°06' W; 700 m.a.s.l.). The experimental treatments included untouched flowers, manual self-pollination, emasculation and cross-pollinations. Each treatment used 40 flowers (eight plants; eight inflorescences) for the plants collected at the SJ, and 45 flowers (nine plants; nine inflorescences) for individuals from the Picinguaba region. For each inflorescence, five flowers per treatment were used. These treatments were applied randomly on each inflorescence, using flowers on the first day of anthesis. The values of fruit set (self- and cross-pollinations) were compared using a *t*-test for independent samples with STATISTICA 6.0 (StatSoft Inc. 2003).

Natural fruit set (open pollination) was recorded at both study sites. In the SJ, 23 plants (23 inflorescences; 623 flowers) we sampled, while in Picinguaba, 14 individuals (14 inflorescences; 504 flowers) were studied. In both regions, the censuses were performed in March 1999. Fruit set was recorded when fruits were dehiscent. The number of flowers sampled varied and depended on the total production in each year.

RESULTS

Habitat, phenology and plant features

Epidendrum secundum is either a lithophyte or a terrestrial herb in the SJ, whereas in the Picinguaba region it grows on acid soils, occurring in disturbed areas along roadsides. At both study sites, isolated individuals occasionally occur as epiphytes.

In both study areas *E. secundum* flowers all year round, but presents a main flowering peak between September and January. Inflorescences of *E. secundum* can be observed simultaneously with buds, open flowers and fruits. Flower opening is diurnal and each flower lasts about 7 days.

Epidendrum secundum is characterized by slender (cane-like) stems with several elliptic–lanceolate and coriaceous distichously disposed leaves. Each stem produces a single terminal, long pedunculate and corymbose inflorescence with up to 130 non-resupinate flowers. Axillary secondary racemes occasionally develop from upper nodes of older peduncles. Sepals and petals are pink, with a large central, white callus on their lip (Fig. 1C and D). Sepals (ca. 14 × 4 mm) are elliptic–lanceolate. Petals measure about 12 × 3 mm and are elliptic. The three-lobed lip is adnate to the entire length of the column and presents a tubular nectariferous chamber (cuniculus) disposed parallel to the ovary. The cuniculus presents numerous papillae covered with a thick cuticle. The column is 3- to 5-mm long and is slender. The anther (1.5 × 1 mm) is green. The pollinarium presents four yellow, laterally flattened pollinia, and a ventral adhesive viscidium.

At Picinguaba, *E. secundum* is sometimes sympatric with *E. fulgens* (Fig. 1L), a very common species occurring in 'restinga' vegetation in South and Southeastern Brazil, which grows on rocks and sandy soils in the Picinguaba region. Individuals of *E. fulgens* sometimes occur among populations of *E. secundum*, along roadsides in this region. In these disturbed areas, we identified dozens of individuals presenting flowers with characteristics in-between both sympatric *Epidendrum* species (see Fig. 1A–L). In these individuals, although the floral dimension seems to be constant (*E. R.* Pansarin, personal observation), the colour of the flowers (sepals and petals), and the form and colouration of the central callus vary from plant to plant (Fig. 1A–L).

Pollinators and pollination mechanisms

In both study areas, SJ and Picinguaba, the floral visitors of *Epidendrum secundum* were diurnal Lepidoptera and bumblebees. At the SJ, an unidentified fly species of the family Bombyllidae was also observed visiting flowers. At both study sites, however, only butterflies acted as pollinators (Table 2), and skippers (Hesperiidae) and species of Nymphaliidae were the main pollinators. Species of other families also acted as pollinators of *E. secundum*: species of Lycaeniidae were documented only in the SJ, and Pieriidae were recorded exclusively in the Picinguaba region (see Table 2). At both study areas, pollinator activity on flowers was recorded between 10:00 and 15:00 h. Visits occurred mainly when direct sunlight reached the inflorescences. A single butterfly generally visited one to three flowers per inflorescence and, although the time interval between visits varied, each insect spent only 2–10 s on a single flower.



Fig. 1. Floral variation observed among populations of *Epidendrum secundum* and *Epidendrum fulgens* of Serra do Japi and Picinguaba. A: Putative hybrid 1. B: Putative hybrid 2. C–D: *E. secundum*. E: Putative hybrid 3. F: Putative hybrid 4. G: Putative hybrid 5. H: Putative hybrid 6. I: Putative hybrid 7. J: Putative hybrid 8. K: Putative hybrid 9. L: *E. fulgens*.

Table 2. Pollinator species and respective number of visits on flowers of *Epidendrum secundum* observed at the Serra do Japi and in the Picinguaba region.

species	visitation number
Nymphalidae, Ithomiinae	
<i>Melinaea luduvica paraiya</i> (Reakirt 1866)	2 ^b
Nymphalidae, Nymphalinae	
<i>Dryadula phaetusa</i> (L. 1758)	1 ^a
<i>Heliconius beskeyi</i> (Mén. 1857)	2 ^a
<i>Heliconius erato phyllis</i> (F. 1775)	3 ^a
<i>Heliconius ethila narcaea</i> (Godt. 1819)	12 ^a
<i>Heliconius sara apseudes</i> (Hbn. 1806)	3 ^b
Hesperiidae, Hesperinae	
<i>Euphyes derasa</i> (Herr. -Sch. 1870)	2 ^a
<i>Decinia decinia antus</i> (Mab. 1895)	2 ^b
<i>Lychnuchus celsus</i> (F. 1793)	4 ^a
<i>Miltomiges cinnamomea</i> (Herr. -Sch. 1869)	19 ^a
<i>Niconiades nikko</i> (Hayward 1848)	2 ^b
<i>Vehilius stietomenes</i> (Butler 1877)	1 ^a
<i>Vettius</i> sp. 1	2 ^a
<i>Vettius</i> sp. 2	1 ^a
<i>Vettius</i> sp. 3	3 ^b
Hesperiidae, Pyrginae	
<i>Gesta gesta</i> (Herr. Sch. 1863)	3 ^a , 4 ^b
<i>Heliopetes arsalte</i> (L. 1758)	2 ^a
<i>Urbanus dorantes</i> (Stoll 1790)	3 ^a , 6 ^b
<i>Urbanus simplicius</i> (Stoll 1790)	1 ^a
Pieridae, Pierinae	
<i>Archonias t. tereas</i> (Godt. 1819)	4 ^b
<i>Phoebis</i> sp.	1 ^b
<i>Phoebis philea</i> (L. 1763)	2 ^b
Lycaenidae, Riodininae	
<i>Eurybia pergaea</i> (Geyer 1832)	1 ^a

^a Serra do Japi.

^b Picinguaba.

These butterflies landed on one (Fig. 2A) or several flowers, depending of their size. Immediately afterwards, they uncoiled their proboscis and probed into the nectariferous chamber (cuniculus). When the insect removed the proboscis from the cuniculus, the pollinarium was attached (Fig. 2A). The pollinarium was attached on the ventral surface of the proboscis (Fig. 2B), mainly on the superior

third. Observations on plants of *E. fulgens* occurring among individuals of *E. secundum* at Picinguaba revealed that both *Epidendrum* species exhibited an identical pollination mechanism. Furthermore, *Phoebis philea*, *Urbanus dorantes* and *Heliconius sara*, species recorded as *E. secundum* pollinators (Table 2), were observed visiting flowers of *E. fulgens*.

At both study sites, bumblebee workers (*Bombus atratus* Franklin, in the SJ, and *Bombus* sp., at Picinguaba) visited flowers of *Epidendrum secundum* in the morning (7:30–10:00 h). During the observation period, no species of *Bombus* were observed removing pollinaria from the flowers. Similarly, at the SJ, the unidentified Bombyllidae fly visited the flowers of *E. secundum* without removing a pollinarium. Some diurnal Lepidoptera species such as *Aeria olenae* Weymer, *Cymaenes gisca* Evans and *Arawacus* sp. also visited the inflorescences of *E. secundum* to suck exudates from the base of floral buds. None of the several inflorescences marked in the field presented nocturnal pollinarium removal.

Breeding system and natural fruit set

The treatments performed revealed that *Epidendrum secundum* is self-compatible, but a pollinator is necessary for pollen transfer. There was no significant difference between the mean fruit set resulting from self- and cross-pollination at the SJ (92.5% for self- and 80% for cross-pollinations; t -test = 1.666, $df = 7$, $P = 0.139$). Conversely, a difference was found between the mean fruit set resulting from self- and cross-pollination at Picinguaba (84.4% and 97.7% for manual self- and cross-pollinations, respectively; t -test = 2.309, $df = 8$, $P = 0.049$). The results of the experimental treatments are summarized in Table 3. Under natural conditions (open pollination), the natural fruit set of *Epidendrum secundum* was low: 7.5% and 4.6% in the SJ and at Picinguaba, respectively (Table 3).

DISCUSSION

Floral variation

At the SJ, the flower morphology and colour are relatively constant among plants. All studied individuals produced rose-coloured flowers with a large and white central callus



Fig. 2. A: A pollinarium removed from *Epidendrum secundum* by *Vettius* sp. 1. B: Detail of the proboscis of *Urbanus dorantes* with a pollinarium of *E. secundum*. Scale bars A = 1 cm, B = 0.5 mm.

location	untouched	self-pollination	emasculated	cross-pollination	open pollination
Serra do Japi	(40/0)	(40/37) 92.5%	(40/0)	(40/32) 80%	(623/47) 7.5%
Picinguaba	(45/0)	(45/38) 84.4%	(45/0)	(45/44) 97.7%	(504/21) 4.6%
total	(85/0)	(85/75) 88.2%	(85/0)	(85/76) 89.4%	(1127/68) 6%

Figures between parentheses indicate the number of flowers/fruits, respectively.

(Fig. 1D). In the Picinguaba region, however, dozens of individuals occurred with flowers whose characteristics were in-between *Epidendrum secundum* and *Epidendrum fulgens*, mainly in terms of colouration and form of the central callus, the main floral characteristic distinguishing species within the '*Epidendrum secundum* complex' (Dunsterville 1979; Dressler 1989). Among the intermediate forms occurring at Picinguaba, some presented characteristics of callus and form of the lip closely related to flowers of *E. secundum* (Fig. 1B–I) whereas the flowers of some plants exhibited great similarities with the callus of *E. fulgens* (Fig. 1J–L) and a few individuals presented a central callus with the characteristics of *E. denticulatum* Barb. Rodr. (Fig. 1A), a common species that occurs in areas of 'cerrado' vegetation in the State of São Paulo. All the variations observed among the populations of *E. fulgens* and *E. secundum* are presented in Fig. 1A–L. The occurrence of individuals of *E. fulgens* among populations of *E. secundum* and evidence of conspicuous variation and intermediate forms observed in flower colouration and callus morphology and colour suggests that floral variation can occur through natural hybridization between both sympatric species, as reported for other orchids (see Van der Pijl & Dodson 1966; Linder 1990; Romero & Carnevali 1990), including species belonging to the '*E. secundum* complex' (Van der Pijl & Dodson 1966; Dressler 1989; Ackerman 1995). According to Arnold (1997) and Harrison (1990), natural hybrids can be defined as the offspring between individuals from two natural populations, based on one or more heritable characters. It has traditionally been believed that hybrids express intermediate characteristics of parental species (Rieseberg & Ellstrand 1993; Rieseberg 1995, 1997).

The existence of putative hybrids between *E. fulgens* and *E. secundum* at Picinguaba is supported by four factors: (i) pollen transfer between the two sympatric species was recorded (see Pollinators and pollination mechanisms); (ii) individuals occurring at Picinguaba region present flowers with intermediate characteristics between the two sympatric *Epidendrum* species; (iii) inter-specific crosses among plants of *E. secundum* and *E. fulgens* produce viable seeds (E. R. Pansarin and L. M. Pansarin, unpublished results) as well as recorded in other species belonging to the '*Epidendrum secundum* complex' (Dressler 1989); and (iv) the occurrence of hybrids between the species of the genus *Epidendrum* belonging to the '*Epidendrum secundum* complex' was also recorded in areas of Central and South America (Van der Pijl & Dodson 1966; Dressler 1989; Ackerman 1995).

Table 3. Reproductive system and natural fruit set (open pollination) of *Epidendrum secundum* in plants from two localities, Serra do Japi and Picinguaba.

Pollinators and pollination mechanisms

As in reports from other localities (Van der Pijl & Dodson 1966), diurnal Lepidoptera are the main pollinators of *Epidendrum secundum* at the SJ and in Picinguaba. In all the Lepidoptera species, pollinaria attachment occurs on the ventral surface of the proboscis (Fig. 2B), mainly on the superior third, as reported for moths that act as pollinators of species of the genus *Angraecum* (Nilsson 1978; Nilsson *et al.* 1985) and butterflies and moths pollinating *Epidendrum paniculatum* Ruiz & Pav. at SJ (Pansarin 2003). *E. secundum* shows low-pollinator specificity, with 23 species acting as pollinators at both study sites (Table 2). Since Lepidoptera rarely return to the inflorescences of *E. secundum* (Pansarin, personal observation), most pollinaria are lost. Nevertheless, in both regions, some skippers, such as *Vettius* spp. and *Urbanus dorantes*, and several species of *Heliconius* and, at the SJ, *Lychnuchus celsus* and *Miltomiges cinnamomea*, frequently returned with pollinaria to flowers of this species. According to Dodson (1962a), *E. secundum* is pollinated by hummingbirds in Central America. Van der Pijl & Dodson (1966) also reported one unidentified species of skipper and *Papilio polyxemes* (Papilionidae) as pollinators of this orchid species in Costa Rica. Yet no hummingbirds or papilionid butterflies were observed visiting flowers of *E. secundum* under natural conditions in either study areas. Mimicry between *E. secundum* and other sympatric species, as is the case of *E. ibaguense* and *E. radicans*, and *Lantana camara* L. and *Asclepias curassavica* L. (Boyden 1980) were not observed among the studied population. *Epidendrum secundum*, therefore, does not produce nectar despite the presence of a nectary-like cuniculus and is apparently pollinated by deceit, as found in *E. ibaguense* and *E. radicans*, which do not produce nectar, being pollinated throughout deceptive mechanisms (Boyden 1980).

Breeding system, fruit set and reproductive isolation

Epidendrum secundum is self-compatible but a pollinator is necessary for pollen transfer, as reported for many Laeliinae species, such as *Laelia* (Stort & Galdino 1984), *Cattleya* (Stort & Galdino 1984), *Pseudolaelia* (Borba & Braga 2003), *Constantia* (Matias *et al.* 1996) and *Epidendrum* (Iannotti *et al.* 1987), but see Adams & Goss (1976), Goss (1977) and Pansarin (2003). The fruit set of *E. secundum* is similar to other self-compatible and non-autogamous orchid species occurring at the SJ and in

Picinguaba (Mickeliunas *et al.* 2006; Pansarin *et al.* 2006; E. R. Pansarin & M. C. E. Amaral, unpublished results). For *E. secundum*, the low rate of fructification observed appears to be related to infrequent visits to flowers, resulting in a strong loss of pollen. At SJ and in Picinguaba, *E. secundum* pollinarium removal was higher than deposition on stigmas (E. R. Pansarin, personal observation). Similarly, *E. paniculatum* was also pollinated by Lepidoptera at the SJ, although most of the removed pollinaria were lost, negatively influencing the reproductive success of this orchid species at this site (Pansarin 2003). As in most Orchidaceae, pollen is packed into pollinia whose removal by an inefficient pollinator results in large pollen loss that may negatively influence the reproductive success of orchid species (Tremblay 1992). The fruit set of some non-autogamous orchids is low as a consequence of deficient pollen transference between plants where the scarcity of efficient pollinators appears to be a limiting factor (e.g. Janzen *et al.* 1980; Schemske 1980; Ackerman & Montalvo 1990; Calvo 1990), although other features, such as inflorescence size, population density, flowering synchronization and flower duration also can exert a strong influence on the reproductive success of animal-pollinated plants (see Tremblay *et al.* 2005, for a review).

The inter-specific treatments involving *E. secundum* reveal that this species is inter-compatible with several species as *E. fulgens*, *E. denticulatum* and *E. aff. ibaguense* (E. R. Pansarin and L. M. Pansarin, unpublished results). Other evidence is that, when back-crossed with their parental species, these putative hybrids also produce viable seed (E. R. Pansarin and L. M. Pansarin, unpublished results). Furthermore, other Laeliinae, such as *Cattleya*, present inter-compatible species (Stort 1983). This can be corroborated by the existence of several hybrids among *Epidendrum* species, *Cattleya* and *Laelia*, not to mention inter-generic crosses, as in *Laeliocattleya*, *Brassocattleya* and *Brassoleliocattleya* that are encountered commercially. These factors reinforce the hypothesis that hybridization between *E. secundum* and *E. fulgens* is perfectly possible when the two species come into contact and share the same pollinators. In Orchidaceae, the occurrence of natural hybridization between such populations is mainly avoided by pre-mating barriers (Van der Pijl & Dodson 1966; Arnold 1997). In orchids, pre-pollination barriers, such as differences in floral morphology, specificity of pollinators and temporal separation of flowering period, are the main factors that guarantee reproductive isolation between sympatric species (Van der Pijl & Dodson 1966; Dressler 1981; Paulus & Gack 1990). When such pre-pollination barriers are strong, hybrids are rare or nonexistent (Linder 1990; Rossi *et al.* 1992; Steiner *et al.* 1994). However, when such barriers are absent or weak, natural hybridization occasionally occurs (Dodson 1962a,b; Van der Pijl & Dodson 1966; Schrenk 1978; Romero & Carnevalli 1990, 1991a,b, 1992; Borba & Semir 1998; Levin 2000). In the State of São Paulo, *Epidendrum secundum* is a common species that occurs on rocks in regions of higher altitude, in natural bare

places at high elevations in semi-deciduous forests and in disturbed areas along roadsides in Atlantic rain forests. In the Picinguaba region, the occurrence of *E. secundum* can probably be related to anthropogenic disturbance. This species occurs only along roadsides and is not found in natural open areas such as 'restinga' vegetation. Thus, the contact between *E. secundum* and *E. fulgens* was probably made possible mainly because of the destruction of the native Atlantic rain forests that acted as a natural barrier. In Picinguaba, the 'restinga' vegetation and forest on steep slopes of the Atlantic rain forest vegetation once formed a continuum. With the destruction of native vegetation due to road construction, several species of plant invaders established themselves along roadsides, including *E. secundum*. Furthermore, most orchids have dry capsular fruits and disperse many thousands of dust-like seeds in the wind over great distances so that they can rapidly colonize open areas (Dressler 1981). These seeds germinate and the plants can establish within parental populations. A similar process of population contact between species of the genus *Catasetum* after the breakdown of natural barriers due to native rain forest destruction promoted the formation of natural hybrids elsewhere (Dodson 1962a).

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