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ScienceDirect

Flora 203 (2008) 474–483

FLORA

www.elsevier.de/flora

Pollen and nectar as a reward in the basal epidendroid *Psilochilus modestus* (Orchidaceae: Triphoreae): A study of floral morphology, reproductive biology and pollination strategy

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Received 2 April 2007; accepted 11 July 2007

Abstract

Psilochilus modestus Barb. Rodr. is a basal epidendroid orchid occurring in both the semi-deciduous and Atlantic rain forests of the state of São Paulo, southeastern Brazil. This species presents a perfect flowering synchrony within populations, since all the mature buds of each plant open simultaneously in the morning hours of the same day. These flowers are available only for 1 day and are pollinated by several species of small solitary and social native bees. These bees exploit both pollen and nectar as a reward. The bees collecting pollen promote a higher fruit set and perform mainly self-pollination while those collecting nectar, which are less numerous, are responsible for an increase in cross-pollinations. *P. modestus* is self-compatible but pollinator dependent. Natural fruit set (open pollination) is low when compared with the numbers obtained under manual self- and cross-pollination. Low fruit set in natural conditions is related to deficient pollen transfer, and pollinator inefficiency seems to be the main factor. Some factors, such as the small amount of nectar produced, the low number of flowers per inflorescence and their availability for 1 day only added to the perfect flowering synchrony seem to be responsible for the increase of cross-pollinations. The offering of both pollen and nectar as a reward can represent a transitional condition in basal Epidendroideae. Based on floral morphology, reward production and pollinator behavior, the relationship of *P. modestus* with the basal and most derived groups within Orchidaceae is discussed.

Keywords: Floral biology; Orchidaceae; Pollination; *Psilochilus*; Reproductive biology; Triphoreae

Introduction

The family Orchidaceae is divided into five subfamilies, including Epidendroideae (Cameron et al., 1999; Freudenstein and Rasmussen, 1999; Freudenstein et al., 2000), the largest subfamily within the orchid family and comprises more genera and species than all the other subfamilies altogether (Dressler, 1993). Epidendroideae is divided into "lower" and "higher" epidendroids. The "lower" epidendroids form a paraphyletic grade. Although the relationships among the "lower" epidendroids are unresolved, Triphoreae, Tropideae and Neottieae form a monophyletic unit (Cameron et al., 1999). *Psilochilus* Barb. Rodr. is included in the tribe Triphoreae (e.g., Cameron et al., 1999; Dressler, 1993; Rothacker, 2006). This genus presents 7–9 terrestrial

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doi:10.1016/j.flora.2007.07.004

species occurring throughout the Greater and Lesser Antilles, Central America and tropical South America (Rothacker, 2006) and includes *Psilochilus modestus* Barb. Rodr., distributed mainly in Atlantic rain forests areas of southeastern Brazil (Miller and Warren, 1996).

According to van der Pijl and Dodson (1966), most orchid species are pollinated by Hymenoptera, which exploit different rewards or are attracted to orchid flowers by deceit. About one-third or more of the species within family Orchidaceae is estimated to be pollinated by deceit (Ackerman, 1986; Nilsson, 1992; van der Pijl and Dodson, 1966). Among the rewards Orchidaceae offer to pollinators, the most common and widespread is nectar (Nilsson, 1992; van der Pijl and Dodson, 1966), which is exploited by several groups of social and solitary bees (see van der Pijl and Dodson, 1966 for a review). Other rewards exploited by bees within the orchid family include fragrance chemicals (volatile oils), edible oils, resins, pseudopollen and food hairs (van der Pijl and Dodson, 1966; Williams, 1982). Pollen as a reward is rare among orchids and is only available in species of the genera *Apostasia* and *Neuwiedia*, both included in the basal subfamily Apostasioideae. The tropical Asian genus *Apostasia* presents two anthers forming a cone-like structure very similar to those of the buzz-pollinated flowers of *Solanum* (Solanaceae). Vogel (1981) speculated *Apostasia* offers pollen as a reward based on flower morphology, since no direct observations were made on flowers. On the other hand, the pollen of *Neuwiedia* flowers is actively collected by *Trigona* bees (Inoue et al., 1995; Kocyan and Endress, 2001; Okada et al., 1996). No direct pollen collection has been recorded for the most derived subfamily within Orchidaceae. Gregg (1991b) reports pollen collection in the nectarless Vanilloideae *Cleistes divaricata* (L.) Ames and *Cleistes bifaria* (Fernald) Catling & Gregg. Therefore, no direct observation of pollen collection from anthers has been recorded for these species. In *Cleistes divaricata* and *Cleistes bifaria* naïve bees clean pollen grains deposited on their body parts (Gregg, 1991b). A similar process of pollen collection is presented by the vanilloid *Cleistes libonii* (Rchb. f.) Schltr. (as *Cleistes macrantha* (Barb. Rodr.) Schltr.) in southeastern Brazil. In *Cleistes libonii*, bumblebees visit the flowers and collect the nectar secreted by two nectariferous glands at lip base. When the bees abandon the flowers, pollen grains are deposited on their scutum. These bees sometimes clean the pollen deposited on their body and pack it into their hind tibial corbicula (Pansarin, 2003a). Species belonging to the vanilloid tribe Pogonieae (Gregg, 1989; Mehrhoff, 1983; Pansarin, 2003a; Thien and Marcks, 1972) present soft pollen (i.e., pollen not agglutinated into pollinia), as is also the case with members of tribe Triphoreae (Rothacker, 2006) and the genera of Apostasioideae (Inoue et al., 1995; Kocyan and Endress, 2001; Okada et al., 1996).

According to Rothacker (2006), no significant work on pollination has been published for Triphoreae, except for *Triphora trianthophora*, which is the most extensively studied member of the tribe as a whole. The floral structure of genera within the tribe indicates that flowers are either pollinated by small bees or autogamous (Dressler, 1981, 1993). According to Dressler (1981), self-pollination is common in populations of *Psilochilus physurifolius* (Rchb. f.) Løjtnant. Similarly, Ackerman (1995) suggested that the flowers of *P. macrophyllus* Ames are cleistogamous because no open flowers had ever been observed. Studies on the reproductive system of *Psilochilus* are thus lacking. Furthermore, data about biotic pollination have never been reported for species of this genus. The present work presents a study of the floral morphology, phenology, pollination mechanisms and reproductive biology of *P. modestus* based on two distinct populations growing in different types of vegetation (Atlantic rain forest and semi-deciduous mesophytic forest) in southeastern Brazil. This paper discusses the effects of flowering synchronization on the pollinators' environment, pollinator efficiency in pollen transference and the effects of not removing pollen in packets (pollinia) on fruit set. Based on floral morphology, rewards and pollinator behavior, we also discuss the relationship of *P. modestus* with the basal and most derived groups within Orchidaceae, in an evolutionary context.

Material and methods

Study sites

Fieldwork was carried out in a semi-deciduous forest in the Serra do Japi, close to the city of Jundiaí and in the Atlantic Rain Forest of Picinguaba, north of the city of Ubatuba, both in the state of São Paulo, Brazil. The Serra do Japi is about 350 km away from Picinguaba. In the Serra do Japi (23°11′S, 46°52′W; elevation 700–1300 m), annual rainfall is about 1.500 mm, and annual average temperature circa 17.5 °C (Pinto, 1992). The Serra do Japi is characterized by a semi-deciduous mesophytic forest of medium altitude with occasional rocky outcrops (Leitão-Filho, 1992). In the Picinguaba region (23°33′S, 45°04′W; elevation 0–50 m), in summer, rainfall reaches about 2.600 mm, and average temperature about 21 °C (Nimer, 1977). Picinguaba is characterized by an evergreen Atlantic rain forest on steep slopes and "restinga" vegetation (Suguio and Martin, 1990).

Phenology and plant features

To check the plant phenology, both study areas were visited monthly from January 1998 to March 2001.

Morphological features of fresh flowers collected in both study sites (Serra do Japi and Picinguaba) were recorded using a binocular stereomicroscope. For the anatomical studies, flowers of *P. modestus* were fixed in FAA 50%. Their lips were dehydrated in an ethanol series and then embedded in glycol methacrylate. Cross-sections were obtained using a microtome. The 9–12-μm thick sections were stained with toluidin blue (Sakai, 1973).

Plant vouchers are deposited at the UEC herbarium, Universidade Estadual de Campinas. Vouchers numbers are: *E.R. Pansarin 132*, for Serra do Japi, and *E.R. Pansarin s.n.* (UEC 105374), for Picinguaba.

Pollinators and pollination mechanisms

In both habitats field visits were undertaken to observe and record the pollination process and to capture pollinators for later identification. Both study regions were visited during the 1998–2000 flowering periods, totaling 38 and 36 observation hours in the Serra do Japi and at Picinguaba, respectively. In both regions, the daily observation period was between 6:30 and 18:00 h. Insects were captured after abandoning the flowers and then identified and deposited at the Natural History Museum of the Universidade Estadual de Campinas (ZUEC).

Breeding system and reproductive success

The reproductive system of *P. modestus* was investigated in the field at both study regions. Experimental treatments included untouched flowers, manual self-pollination, emasculation and cross-pollination. These treatments were randomly applied to each inflorescence, using flowers on their first day of anthesis. The number of flowers used varied between treatments and study site, depending on flower availability.

Natural fruit set (open pollination) was recorded in both study areas. Sampling comprised 34 inflorescences (34 plants) in the Serra do Japi and 41 inflorescences (41 plants) in Picinguaba. In both regions, the censuses were realized when fruits were dehiscent.

Results and discussion

Phenology and plant features

P. modestus is a terrestrial herb growing in the dense burlap layer within the forest of the Serra do Japi and in Picinguaba. In the Serra do Japi, the flowering period occurred from December to March and fruits were dehiscent between June and July. In Picinguaba, the flowering period occurred from January to May and fruits were dehiscent in August. Flowers opened in the morning hours (between 6:00 and 6:30 h), and each one lasted flowering about 12 h.

P. modestus is a gregarious species presenting flower synchronization that occurs in irregular intervals from 7 to 21 days. In this flowering synchronization all mature buds of all individuals within a population opened simultaneously in the morning hours of the same day. Similar flowering peaks with an identical synchrony of flower anthesis as observed in *P. modestus* have been reported for several other basal epidendroid genera (see Dressler, 1993), for *Dendrobium crumenatum* Sw. (Seifriz, 1923), and members of the vanilloid genus *Cleistes* (Pansarin, 2003a, 2004). In *C. libonii* (Rchb.f.) Schltr., *C. pusilla* Pansarin and *T. trianthophora* (Sw.) Rydb., the flowering synchronization seems to be mediated by water (Medley, 1979; Pansarin, 2003a, 2004). As *P. modestus*, *T. trianthophora* belongs to the tribe Triphoreae (Cameron et al., 1999; Dressler, 1993; Rothacker, 2006). Nevertheless, in the case of *P. modestus*, no relation between precipitation and synchronization of flower opening could be inferred because flowering occurred in both wet and dry days in both study areas (Serra do Japi and Picinguaba). Rothacker (2006) reported that *T. trianthophora* is thermoperiodic, that is, it requires a number of cool evenings followed by significantly warmer days prior to blooming. During the period preceding flowering, characteristically nodding buds become erect. All flowers in a population open early in the morning and, as is the case with *P. modestus*, last approximately 12 h and then drop unless fertilization occurs. Synchronous or gregarious blooming, which is characteristic of the tribe Triphoreae, also occurs in many terrestrial orchids. Since single individual flowers are rarely pollinated, synchronous blooming increases the likelihood of cross-pollination by exposing several plants in a population to pollinators at the same time (Rothacker, 2006).

Plants of *P. modestus* are erect and up to 54 cm tall. Their slender stem is green or purple, cylindrical in cross-section, fleshy and coated with white to brown hairs. Leaves are about 7.5 cm long and 4.5 cm wide, alternate, obliquely disposed along the stem and present an invaginant sheath. They are shiny green on their adaxial surface, elliptic-lanceolate with green abaxial surfaces in the populations of Picinguaba, and oval-lanceolate with green-purple abaxial surfaces in the populations of the Serra do Japi.

The flowers of *P. modestus* are resupinate and arranged in a terminal raceme with up to six flowers. Flowers have a mainly white perianth, present a green ovary and a pedicel covered by a foliaceous and persistent bract. The dorsal sepal is symmetrically oblanceolate. The lateral sepals are falcate. All sepals are about 2 cm long and 0.5 cm wide, white-green, fleshy and free. Petals are spathulate (2 cm long), membranaceous, and white-hyaline. Lip (2 cm long) is obovate,

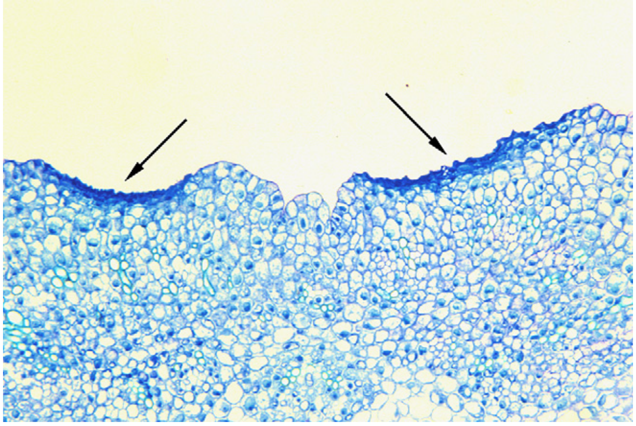

Fig. 1. Cross-section of a lip base of *Psilochilus modestus* showing epidermal nectaries.

3-lobate, and white; its apical lobe is pink with white diagonal stripes. The mid-portion of the lip presents white or yellow longitudinal lines. The lip base presents two epidermal nectaries (Fig. 1). Nectar is secreted before flower opening, as has also been reported for *C. libonii* (Pansarin, 2003a). In *T. trianthophora*, nectar, which is stored at lip base, is apparently secreted by ridges on the column base (Medley, 1979). According to Catling and Catling (1991), however, the attractants and floral rewards of *T. trianthophora* have to be studied in more details.

The column of *P. modestus* is convex, slim and about 2 cm long. Anther cap is white and firmly attached to a thick filament and remains attached to the column after the pollinarium has been removed, as reported for other basal groups within Orchidaceae (Dressler, 1993). Pollinarium presents two elongated and bipartite white pollinia (i.e., in tetrads) with a mealy consistency (Dressler, 1993; Pansarin, 2000). The ventral surface of the pollinarium presents a hyaline and rudimentary U-shaped viscidium. The stigma is oval and about 4 mm long and 2.5 mm wide. A thick rostellum separates the stigma from the pollinarium. For more details on flower morphology and illustrations see Pansarin (2000) and Hoehne (1940).

Flowers of *P. modestus* release a sweet fragrance mainly in the warmest hours of the day. Produced by epidermal papillae on the adaxial surface of the lip (Pansarin, 2000), this fragrance is a mixture of several mono- and sesquiterpenes and aromatic compounds (Reis et al., 2004). Among these compounds is geranial, a common substance in pollen fragrances (Dobson, 1994; Reis et al., 2004).

Pollinators and pollination mechanisms

In both study areas, flowers of *P. modestus* were visited by a variety of insects, including wasps, butterflies, flies, beetles and several species of small solitary and social native bees. However, only the social and solitary bees actually pollinated this orchid specie at both study sites. The floral visits started soon after flower opening (about 6:30 h) and stopped in late afternoon, at about 18:00 h.

As a floral reward, the flowers of *P. modestus* offer pollen as well as nectar, which are exploited distinctly by each pollinator species. Nevertheless, the exploitation of both rewards by a single bee species sometimes occurs, as is the case with *Augochlora* sp. (Halictidae), at Serra do Japi. Pollinators, number of visits and the floral reward exploited by each bee species at both study sites are summarized in Table 1. Visiting time depended on each bee species and on the reward exploited, and varied from a few seconds (bees taking nectar) to minutes (bees collecting pollen). The pollination mechanisms differed markedly depending on whether a bee collects nectar or pollen. When they collect pollen, both solitary and social bees adopted a supine feeding posture. To achieve this position, they landed directly on the flower column. They seized it with their median and posterior legs and ventral portions of their thorax contacted pollinarium (free tetrads). They collected the tetrads with their anterior and median legs, deposited them on their thoracic hairs before transferring them to their tibial corbiculae. The position these bees adopted to collect pollen from *P. modestus* is similar to that described as supine position for *Viola* species (Freitas and Sazima, 2003). In such a position, the legs or ventral parts of bee's body touch the stigmatic surface and usually self-pollinate flowers. Pollen collection in flowers of *P. modestus* permitted that minute bees, as *Plebeia droryana* Friese (Fig. 2A), acted as pollinators of this orchid species. *P. droryana* was responsible for most

Table 1. Pollinators of *Psilochilus modestus*, visitation frequencies and reward exploited by each hymenopteran species at both study sites, Serra do Japi and Picinguaba

Specie	Frequency	Reward
Halictidae		
Halictidae sp. 1[a]	2[a]	Nectar[a]
Augochlora sp.[a]	5[a]	Nectar/pollen[a]
Apidae (Meliponini)		
Melipona sp.[a]	2[a]	Nectar[a]
Plebeia droryana Friese[a]	64[a]	Pollen[a]
Trigona spinipes Fabricius[a,b]	8[a]/41[b]	Pollen[a,b]
Trigona sp.[b]	4[b]	Pollen[b]
Apidae (Osirini)		
Osiris sp.[b]	3[b]	Nectar[b]
Pompilidae		
Pompilidae sp.[b]	7[b]	Nectar[a]

Frequency refers to the visitation numbers documented in the flowering period from 1998 to 2000.

[a] Serra do Japi.

[b] Picinguaba.

Fig. 2. *Psilochilus modestus*: (A) *Plebeia droryana* collecting pollen directly from the pollinarium. Note another bee flying with pollen of *P. modestus* in its tibial corbiculae; (B) *Trigona spinipes* collecting pollen; (C) unidentified Halictidae species taking nectar at lip base; (D) *Augochlora* sp. abandoning the flower after taking nectar at lip base; (E) *Augochlora* sp. collecting pollen; (F) *Salpingogaster* sp. eating pollen. Scale bars = 1 cm.

self-pollinations of *P. modestus* in the Serra do Japi (Table 1). *Trigona spinipes* Fabricius (Fig. 2B) also collected pollen from flowers of *P. modestus*, made most visits, and performed self-pollinations in Picinguaba (Table 1). As reported here for *P. modestus*, *Trigona* bees are very important in the pollination of several orchid species (e.g., Dressler, 1981; Inoue et al., 1995; Pansarin and Amaral, 2006; van der Pijl and Dodson, 1966; Singer and Cocucci, 1999), and also acted as effective pollinators of *Neuwiedia* species that offer pollen as a reward (Inoue et al., 1995; Kocyan and Endress, 2001; Okada et al., 1996). The offering of pollen as a reward is extremely rare in Orchidaceae (see van der Pijl and Dodson, 1966; Williams, 1982) and direct pollen collection from anthers was only reported for the genus *Neuwiedia*, included in the basal subfamily Apostasioideae (Inoue et al., 1995; Kocyan and Endress, 2001; Okada et al., 1996). Gregg (1991b) and Pansarin (2003a) reported pollen collection in vanilloid orchids belonging to the genus *Cleistes*. Nevertheless, in *Cleistes*, the free pollen monads are not collected directly from the anthers. The bees collect the pollen deposited on their body surface after abandoning the flowers where they have taken nectar (Pansarin, 2003a) or to which they were attracted by deceit (Gregg, 1991b).

Osiris sp. (Picinguaba), *Melipona* sp. and some species of Halictidae bees (Serra do Japi; table 1) took nectar from flowers of *P. modestus*. Halictidae bees are important pollinators of other orchid flowers in many regions (Catling, 1983; Goss, 1977; Mehrhoff, 1983; van der Pijl and Dodson, 1966; Pansarin, 2000; Pansarin and Amaral, 2006; Singer and Cocucci, 1999), including *T. trianthophora* in North America, which is pollinated by *Augochlora pura* (Medley, 1979) and *Halictus quadrimaculatus* (Lownes, 1920). Although Rothacker (2006) reported capsules with a high level of seed formation in plants of *T. trianthophora*, no pollinators were observed in Florida. Pollination by parasitic bees of the genus *Osiris* sp. has been observed in the basal epidendroid genus *Palmorchis* in Panama (Dressler, 1983). When bees visited flowers of *P. modestus* to collect nectar, the pollination mechanism was typical of the other bee-pollinated orchids (see van der Pijl and Dodson (1966) and Van der Cingel (2001) for reviews). The bee first landed on the lip and, oriented by yellow or white nectar guides, entered headfirst into the floral tube formed by the labellum and the other two petals, forcing its way underneath and past the anther, rostellum and stigmatic surface. Bees get to the lip base where a small drop of nectar secreted by epidermal nectaries accumulates. After taking this nectar, the bees abandon the flower contacting the rudimentary viscidium with their upper thorax (scutum), where pollinarium is deposited. However, pollen is usually not transported as an intact pollinarium, but as a loose mass of tetrads. A similar mechanism of pollen deposition in masses on the bee body has been described for several basal epidendroid (e.g., Dressler, 1981, 1983, 1993; Thien and Marcks, 1972) and vanilloid orchids (Gregg, 1991a; Mehrhoff, 1983; Pansarin, 2003a; Thien and Marcks, 1972). This strategy allows that more than one bee receive pollen loads from a single flower (Gregg, 1991a; Mehrhoff, 1983; Pansarin, 2003a). Furthermore, the strategy of dispensing tetrad masses can reduce pollen loss when the flowers are visited by an inefficient pollinator. Since in most orchid species the pollen is aggregated into pollinia and transported as a unit by pollinators (Dressler, 1981, 1993; Freudenstein and Rasmussen, 1997), their removal by an inefficient pollinator results in a large pollen loss, which may influence negatively the reproductive success of the species (Tremblay, 1992).

In both studied areas, bees taking nectar resulted in a great increase in cross-pollination because they visited all the flowers available within the population. Bees taking nectar also deposited a greater quantity of pollen grains on the stigma, covering all the stigmatic surfaces, in a similar way to that described for some vanilloid orchids (Gregg, 1991a; Mehrhoff, 1983; Pansarin, 2003a). Contrastingly, when bees collected pollen from flowers, only a few tetrads were deposited on the stigma, promoting fructification. As a consequence, a greater quantity of seeds per fruit (Pansarin, pers. obs.) and possibly a greater genetic variability occurred when bees took nectar, as compared with insects collecting pollen. Self-pollinations (performed mainly by bees collecting pollen) can therefore be an important strategy to promote fructification, when pollinators are scarce or infrequent, as reported here for *P. modestus* in both study sites. According to Tremblay et al. (2005), low pollination frequency and pollen quantity may limit seed production in orchids, but in self-compatible plants poor quality pollen resulting from self-pollination can also reduce seed production.

In the populations of *P. modestus*, the production of few flowers per inflorescence added to the flowering synchrony, the fact that the flowers last only 1 day and the small quantities of nectar produced by a single flower are responsible for a grater occurrence of cross-pollination within populations. As reported here for *P. modestus*, flowering synchrony and the production of few short-lived flowers per inflorescence are important strategies favoring cross-pollinations of *T. trianthophora* in North America (Medley, 1979) and vanilloid *C. libonii* in southeastern Brazil (Pansarin, 2003a).

In both study areas, syrphid flies (*Salpingogaster* spp.) were recorded eating pollen in flowers of *P. modestus* (Fig. 2F). In the Serra do Japi, two species of butterflies (*Heliconius ethila narcacea* F. and *H. erato phyllis* Godt.; Nymphalidae, Nymphalinae) were recorded taking nectar in flowers. Nevertheless, the syrphid flies never touched the stigmatic surface nor did the butterfly proboscides contact the flower reproductive structures.

Breeding system and reproductive success

The treatments performed in both study areas revealed that *P. modestus* is self-compatible but depends on pollen vectors for pollination. Fruit set of *P. modestus* in manual self-pollinated flowers was

90.5% and 74% at Serra do Japi and Picinguaba, respectively. Fructification in cross-pollinated flowers was 90.2% and 82.5% at Serra do Japi and Picinguaba, respectively. No fruits were formed on untouched or emasculated flowers. The results of the experimental treatments are summarized in Table 2.

The production of fruits per inflorescence is still the most used parameter to determine the reproductive success in the orchid family (e.g., Ackerman, 1989; Ackerman and Montalvo, 1990; Montalvo and Ackerman, 1987; Zimmerman and Aide, 1989). The natural fruit set of *P. modestus* was low when compared with that of each inflorescence in artificial self- and cross-pollinations (Table 2). In experimental conditions, an increase of fruit set occurs when compared with the natural condition of several orchid species and seems to be the most common condition among orchids pollinated by biotic vectors (e.g., Ackerman and Montalvo, 1990; Ackerman and Oliver, 1985; Montalvo and Ackerman, 1987; Zimmerman and Aide, 1989), which include epidendroid orchids occurring in the study area (e.g., Mickeliunas et al., 2006; Pansarin, 2000, 2003b; Pansarin and Amaral, 2006). The fruit set of non-autogamous orchids, as is the case of *P. modestus*, is low as a consequence of a deficient pollen transference between plants where scarcity of efficient pollinators seems to be a limiting factor (e.g., Ackerman and Montalvo, 1990; Bierzychudek, 1981; Burd, 1994; Calvo, 1990; Janzen et al., 1980; Schemske, 1980; and references therein). However, other factors can exert a strong influence on the reproductive success of orchid species, such as phenology, inflorescence size, habitat, plant density, population size and temporal variation (see Tremblay et al. (2005) for reviews). The regulation of maternal investment occurs through abortion of flowers and immature fruits (Stephenson, 1981), as occurred in self- and cross-pollinations of *P. modestus* in both study sites. This may also be a mechanism to regulate the seed quality (e.g., Bookman, 1984). The hypothesis that resources are the ultimate limiting factor in angiosperm reproduction has gained widespread acceptance because levels of fruit maturation remain unchanged following supplementary pollination, and the experimental reduction of resource availability causes elevated levels of fruit abortion (see Tremblay et al., 2005). Thus, according to this view, the variation in reproductive success should be closely tied to the severity of resource constraints. Flowers that fail to become fruits are not always wasted as they may function to enhance plant fitness through pollen donation. Furthermore, fruit set varies according to the pollinator group (Tremblay et al., 2005).

Evolutionary implications

Most epidendroid orchids are characterized by the presence of fully incumbent anthers, hard pollinia, thickened stems, and in most of the genera, epiphytic habit (Cameron et al., 1999; Dressler, 1993). However, as observed here for *P. modestus*, other genera belonging to the tribe Triphoreae are included within the epidendroid orchids but lack such synapomorphic features (Cameron et al., 1999). *Psilochilus* presents a set of characters considered to be plesiomorphic within Epidendroideae, as an anther cap united to the column, like Apostasioideae and Vanilloideae (Cameron et al., 1999; Dressler, 1993), gregarious flower synchrony, as has also been reported for some *Cleistes* species (Pansarin, 2003a, 2004) and soft pollen, as occurs in the most basal subfamilies within Orchidaceae (i.e., Apostasioideae, Cypripedioideae and Vanilloideae (Cameron, 2004; Cameron et al., 1999; Freudenstein et al., 2004). The evidence of pollen use as a reward and the presence of epidermal nectaries lead to the idea of a transitional change in one or another way, from nectar to pollen or from pollen to nectar, as has been recently reported in Violaceae (Freitas and Sazima, 2003). We are not able to specify in which way this transition took place because data on pollination are scarce within Triphoreae (Rothacker, 2006) and other basal epidendroid orchids. In contrast to other genera within the most derived Epidendroideae that present sectile and hard pollinia (Cameron, 2004; Cameron et al., 1999; Dressler, 1993; Freudenstein et al., 2004) and offer mainly nectar or other floral resource (e.g., edible oil, fragrances and food hairs; van der Pijl and Dodson, 1966; Williams, 1982), the most basal genera within the subfamily present tetradic pollen, which is collected by several groups of native bees in *P. modestus*, suggesting

Table 2. Total flower number and fruit set in each treatment and *Psilochilus modestus* and fruit set in natural conditions in both study regions: characters between parentheses are the number of flowers/number of fruits, respectively

Region	Untouched	Manual self	Emasculation	Cross poll.	Open poll.
Serra do Japi	(55/0)	(42/38) 90.5%	(40/0)	(41/37) 90.2%	(113/22) 19.5%
Picinguaba	(7/0)	(27/20) 74%	(9/0)	(23/19) 82.5%	(126/3) 2.4%
Total	(62/0)	(69/58) 84%	(49/0)	(64/56) 87.5%	(239/25) 10.5%

that, in Epidendroideae, this transition occurred from pollen to nectar flowers. Our assumptions are based on the following evidences: (1) scanty nectar production by two epidermal nectaries at lip base; (2) basal epidendroids, as the main subfamilies within the orchid family (i.e.; Apostasioideae, Cypripedioideae and Vanilloideae), present loose pollen (Cameron, 2004; Cameron et al., 1999; Freudenstein et al., 2004), but only in Apostasioideae (Inoue et al., 1995; Kocyan and Endress, 2001; Okada et al., 1996) and in lower Epidendroideae, therefore pollen grains are directly collectable from the anthers. Although members of subfamilies Cypripedioideae and Vanilloideae also present free (monadic) and friable pollinia, respectively, their collection is not directly possible because the pollen grains are inaccessible to floral visitors (Dressler, 1993). In the vanilloid genus *Cleistes*, however, indirect pollen collection has been recorded; i.e., bees clean themselves and place free pollen grains in their tibial corbiculae (Gregg, 1989; Pansarin, 2003a).

As pointed out by Dressler (1993), pollen in monads, as found in the most basally diverging orchid genera (*Apostasia* and *Neuwiedia*), is the primitive condition and has evolved several times in the evolution of orchid family. Shifts between monads and tetrads have occurred several times among basal subfamilies in Orchidaceae (Johnson and Edwards, 2000). Sectile and hard pollinia seem to be apomorphic within Orchidaceae and have been used to delimit groups within the family (Freudenstein and Rasmussen, 1997). The occurrence of sectile pollinia arose at least four or five times in the evolution of the family (Burns-Balogh and Funk, 1986; Dressler, 1993).

Acknowledgments

The author thanks G.J. Shepherd, M. Sazima, V. Bittrich and S. Buzato for their valuable suggestions; Alain François for improving the English; Núcleo Picinguaba, Instituto Florestal, Base Ecológica da Serra do Japi and Guarda Municipal of Jundiaí for granting permission for field work. This study is part of a Master thesis by the first author for the post-graduation course in Plant Biology at the 'Universidade Estadual de Campinas' (São Paulo, Brazil). Essential financial support was provided by CNPq and FAPESP (Grant 98/05097-0).

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