The Pollination Mechanism of Three Sympatric *Prescottia* (Orchidaceae: Prescottinae) Species in Southeastern Brazil

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The pollination biology of three *Prescottia* (Orchidaceae: Prescottinae) species was studied in Picinguaba, São Paulo State, south-eastern Brazil. Plants are self-compatible but pollinator-dependent and offer nectar as the only reward for pollinators. *Prescottia plantaginea* Lindl. and *Prescottia stachyodes* (Swartz) Lindl. are pollinated by pyralid moths (Lepidoptera: Pyralidae). Moth-pollination is reported for the first time in the genus *Prescottia* and the subtribe Prescottinae. Pollination by halictid bees (Halictidae) is confirmed for *Prescottia densiflora* Lindl. The pollination mechanism is the same regardless of pollinator-type: pollinaria are fixed on the ventral surface of the insect's proboscis through the pad-like viscidium and are removed when the insect leaves the flower. A pollinarium-carrying insect visiting another flower will brush the stigmatic surface and leave clumps of pollen, thus effecting pollination. *Prescottia stachyodes* is protandrous, a feature that increases the chances of cross-pollination. As far as we know, this is the first report of protandry in the genus *Prescottia* and also the subtribe Prescottinae.   © 2001 Annals of Botany Company

Key words: *Prescottia*, Orchidaceae, orchids, Prescottinae, moths, Pyralidae, bees, Halictidae, flower morphology, protandry.

INTRODUCTION

The neotropical orchid genus *Prescottia* Lindl. contains about 60 species, 30 of which have been recorded in the Brazilian orchid flora (Hoehne, 1945; Dressler, 1993). *Prescottia stachyodes* (Swartz) Lindl. has a notably wide distribution, occurring from north-eastern Argentina to Florida (USA) (Hoehne, 1945; Ackermann, 1995; Correa, 1996).

There are only a few reports of pollination biology for the subtribe Prescottinae. Wasps and flies have been reported to pollinate *Myrosmodes cochleare* (Berry and Calvo, 1991) and Dressler (1993) suggested that the reddish-flowered *Porphyriostachys pilifera* may be pollinated by hummingbirds. Ackermann (1995) suggested the possibility of autogamy in plants of *Prescottia oligantha* (Swartz) Lindl., *P. pellucida* Lindl. and *P. stachyodes* (Swartz) Lindl. occurring in Puerto Rico and the Virgin Islands. More recently, Singer and Cocucci (1999) reported the pollination mechanism in *Prescottia densiflora* Lindl. and recorded halictid bees and, to a lesser degree, syrphid flies as pollinators. During our studies on the reproductive biology of south-east Brazilian orchids, we studied the pollination biology of three *Prescottia* species occurring sympatrically in the Brazilian rainforest. The aims of the present contribution are: (1) to broaden our knowledge on the pollination biology of South American *Prescottia* species; and (2) to highlight the functional flower morphology of this noteworthy orchid genus.

MATERIALS AND METHODS

Studies were conducted in Picinguaba (Parque Estadual da Serra do Mar, Ubatuba, São Paulo State, Brazil) approx. 23° 22′S and 44° 55′W. Annual rainfall reaches 2600 mm and the annual average temperature is approx. 26 °C (Nimer, 1977). The climate is tropical-humid (Koeppen, 1948) and without a well-defined dry-cold season (Nimer, 1977). The pollination biology of the species studied was documented during several field visits. Phases of anthesis, life span of flowers and other floral characteristics (e.g. fragrance emission) were observed either *in situ* or in cultivated plants used for breeding experiments (see below). Observations made during preliminary fieldwork and also using cultivated plants provided evidence of flower-specific features (particularly the period of fragrance emission) which were used when deciding the observation period for each species (see below).

Prescottia densiflora Lindl. occurs in natural or man-made grasslands. The population studied occurs near the Park base at an altitude of about 40 m a. s. l. The study site is surrounded by Atlantic rainforest ('Mata Atlântica') vegetation. The pollination biology of this species was monitored during six field visits, from 23–24 Jul. 1999 and 2–30 Oct. 1999. About 15 h 30 min were spent in observations, which were made from 0900 to 1500 h.

Prescottia plantaginea Lindl. occurs in stands in grassy or rupicolous open areas. The population studied occurs near a tourist point, known locally as 'Casa da Farinha' at an altitude of about 40 m. a. s. l. The pollination biology of this species was studied by means of six field visits, from 8–14 Aug. 2000. About 15 h 10 min were spent in observations, which were made from 1700 to 2100 h.

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TABLE 1. *Fruit set (%) of three* Prescottia *species following emasculation, self- or cross-pollination, or a control treatment*

	N	Control	Emasculation	Self-pollination	Cross-pollination
P. densiflora	4	0 (0/286)	0 (0/25)	69·2 (18/26)	100 (20/20)
P. plantaginea	4	0 (0/126)	0 (0/32)	48·04 (49/102)	48·1 (25/52)
P. stachyodes	9	0 (0/937)	0 (0/211)	93·7 (209/223)	95·9 (209/218)

Numbers in parentheses represent the number of fruits obtained over the number of flowers used in each treatment. *N*, Number of individuals per species.

χ^2 (self-$\times$ cross-pollination) values; *P. densiflora*: 7·47 (significant, $P = 0.0065$); *P. plantaginea*: 0 (non-significant, $P = 0.99$); *P. stachyodes*: 1·06 (non-significant, $P > 0.5$).

Prescottia stachyodes (Swartz) Lindl. is widespread in the whole Park area, but its pollination biology was studied in a stand near 'Trilha do Mangue', at about 6 m a. s. l. The surrounding vegetation consists of a dwarf (up to 10 m) forest, known locally as 'restinga'. The natural pollination of this species was studied during five field visits, from 28 Jun.–4 Jul. 1999 and from 22–23 Jun. 2000. About 22 h 25 min were spent in observations, which were made from 1700 to 2100 h.

In 2000, the fruiting success (the number of dehiscent fruits divided by the number of flowers produced) was calculated for each species. Twenty inflorescences of *P. densiflora* (totalling 1841 flowers), eight of *P. plantaginea* (totalling 645 flowers) and eight of *P. stachyodes* (totalling 937 flowers) were available.

Flower morphology was studied using fresh and preserved (F. A. A. 50 %) flowers. Drawings were made using a stereo-microscope with a camera lucida attachment. Plant vouchers are deposited at the Herbarium of the Universidade Estadual de Campinas (UEC): voucher numbers: *Prescottia densiflora*, R. B. Singer 99/20; *Prescottia plantaginea*, R. B. Singer 99/09; *Prescottia stachyodes*, R. B. Singer 99/08.

Breeding systems were studied using plants cultivated in a glasshouse, at the Universidade Estadual de Campinas. The number of plants per species and flowers per treatment are summarized in Table 1. Treatments included untouched flowers (control), emasculation, self-pollination, and cross-pollination. Given the peculiar flower morphology (non-resupinate flowers with hidden stigmatic surfaces), the lip (labellum) was removed to expose the stigmatic surface. Pollinaria were removed using entomological pins and brushed against the stigmatic surface. Treatments yielding fruits were compared by pairs, through a χ^2 test ($\alpha = 0.05$, d.f. = 1). When necessary, the Fischer correction was performed. Insect behaviour at flowers was recorded through field notes and photographs. In night-pollinated flowers, insect behaviour was followed with the help of a flashlight, a device which apparently did not disturb insect behaviour. Some insects were captured for later identification. Insect vouchers are deposited at ZUEC (Museu de História Natural da Universidade Estadual de Campinas). Throughout this paper, the taxonomical and morphological concepts of Dressler (1993) are followed.

RESULTS AND DISCUSSION

General plant features

The three species studied share a number of vegetative and floral features: roots are fasciculate and thick; leaves are rosulate, sessile to petiolate; inflorescences are terminal, many-flowered spikes (Hoehne, 1945); flower opening is sequential, from the base of the inflorescence toward its top; flowers are non-resupinate and dome-like (Hoehne, 1945; Dressler, 1993) (Fig. 1A, B, E, F, I and J); the rostellum is short (Fig. 1C, G and K) and the anther holds a pollinarium made up of four clavate, granulate, yellowish pollinia, reduced caudicles and a terminal oblong, pad-like viscidium (Rasmussen, 1982, Dressler, 1993) (Fig. 1D, H and L); the stigmatic surface is broad, entire to slightly bilobed (Rasmussen, 1982; Dressler, 1993; Singer and Cocucci, 1999) (Fig. 1C, G and K); flowers offer nectar as the only reward to pollinators (Singer and Cocucci, 1999), which is apparently secreted by the two retrorse lobes at the base of the lip (Dressler, 1993); and fruits are capsules bearing a number of minute, dust-like and monoembryonic seeds.

Specific plant features and pollination biology

Prescottia densiflora. Leaves are sessile to attenuate [they were incorrectly referred to as 'petiolate' by Singer and Cocucci (1999)] and broad, but can be absent or deteriorate somewhat during anthesis. The inflorescences are congested and cylindrical, and may bear up to 80 flowers, which are whitish (Fig. 2A and B), sometimes with delicate pink lines in the sepals. A dense tuft of hairs is visible on the inner surface of the lip, above the column (Singer and Cocucci, 1999). The flowers emit a musky fragrance during the sunniest hours (Singer and Cocucci, 1999) and untouched flowers may keep their fresh appearance for 4–7 d.

Our observations confirmed the report of Singer and Cocucci (1999) on the pollination mechanism of *P. densiflora*. Halictid bees of the genera *Augochloropsis* and *Pseudoagapostemon* (Fig. 2A and B) were recorded as pollinators. Bees were recorded at the flowers from 1130 to 1400 h. The bees fixed pollinaria on the ventral surface of their proboscis while probing the flowers for nectar. When

FIG. 1. A–D, Floral features of *Prescottia densiflora*. A, Frontal view of flower. B, Lateral view of flower. C, Column, ventral view. D, Pollinarium. E–H, *Prescottia plantaginea*. E, Frontal view of flower. F, Lateral view of flower. G, Column, ventral view. H, Pollinarium. I–L, *Prescottia stachyodes*. I, Frontal view of flower. J, Lateral view of flower. K, Column, ventral view. L, Pollinarium. STG, Stigmatic surface; V, viscidium.
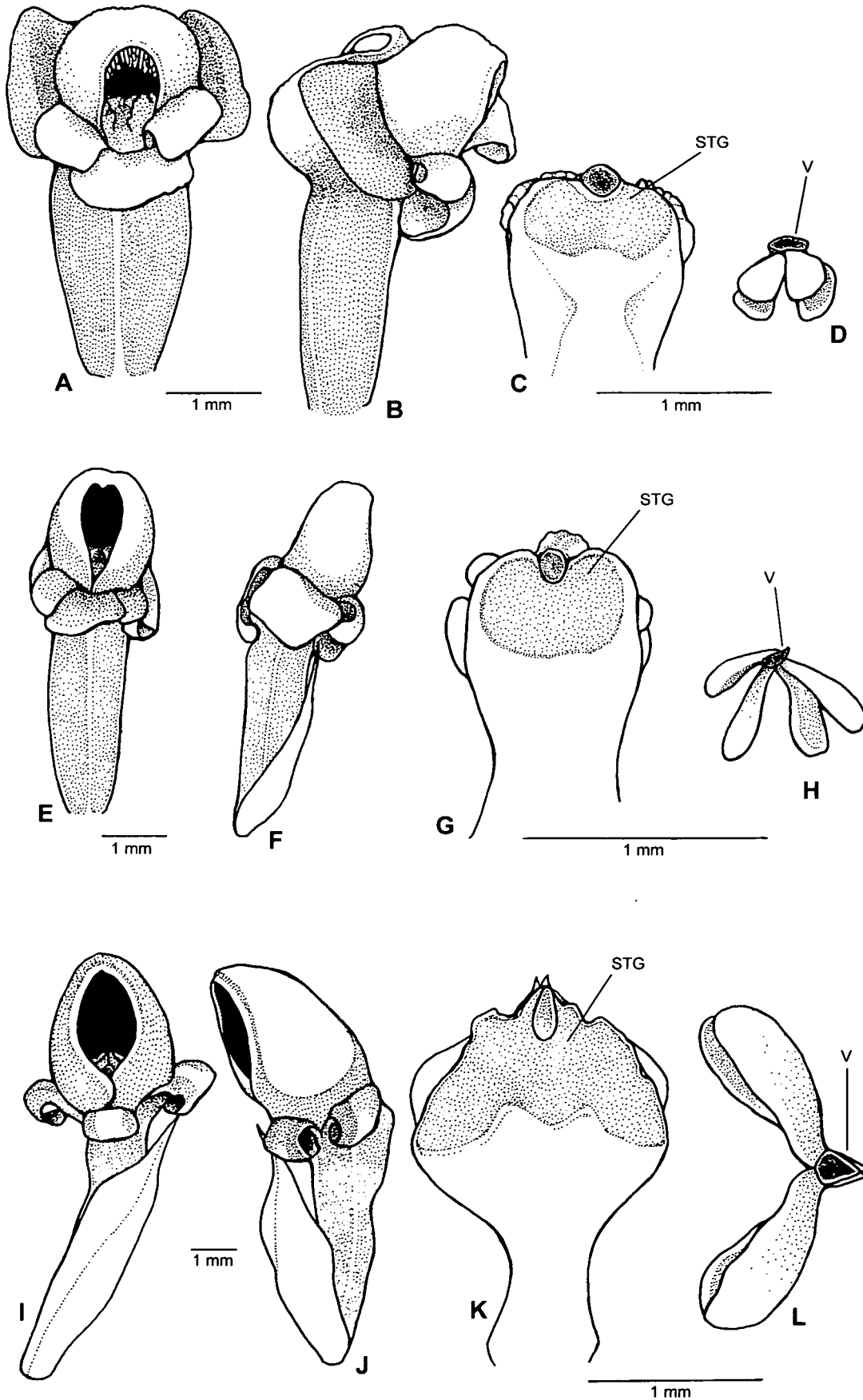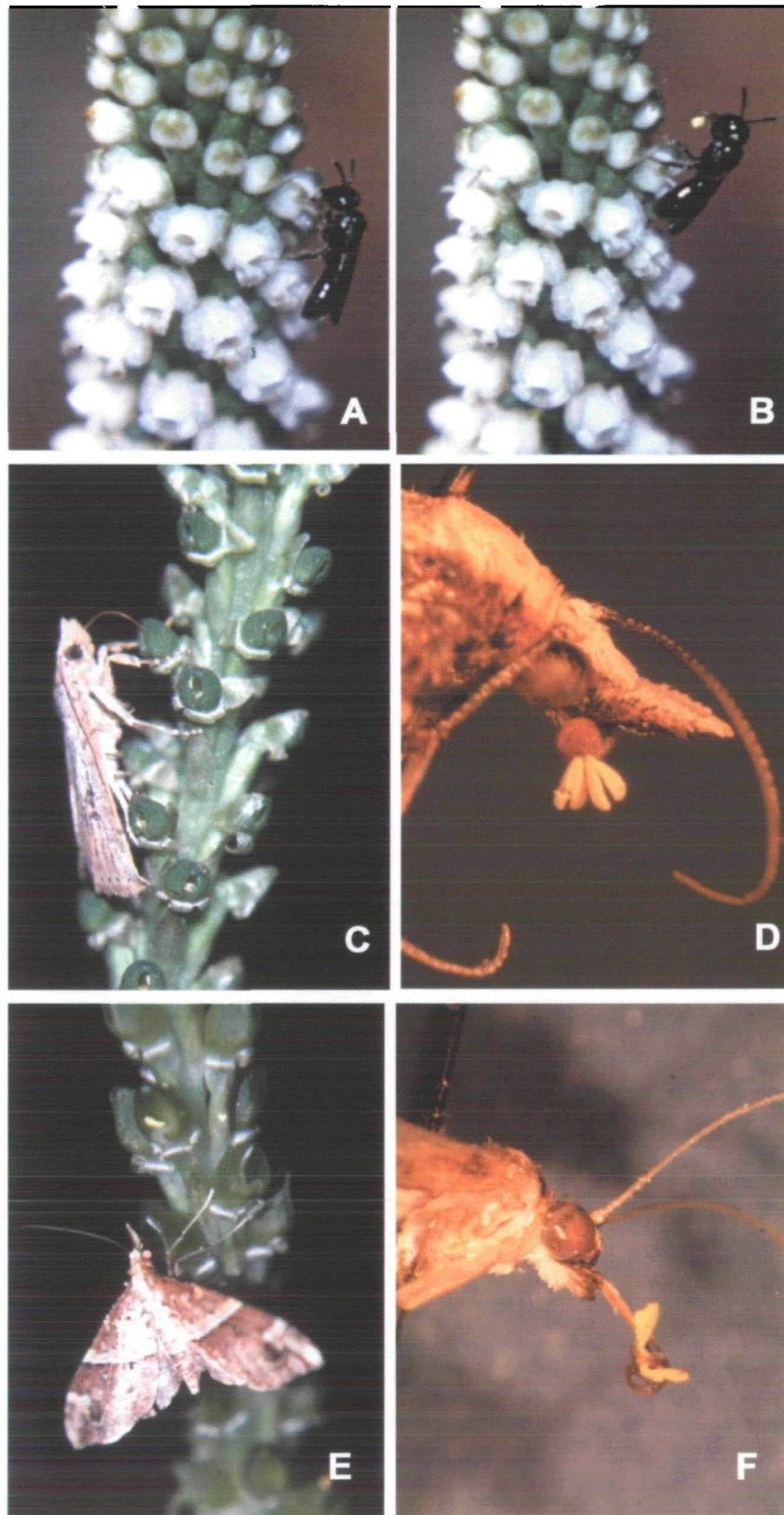
FIG. 2. A and B, Halictid bee visiting *P. densiflora*. A, Bee probing a flower. B, Bee dislodging a pollinarium. C, Pyralid moth probing flowers of *P. plantaginea*. D, Pyralid moth with a pollinarium of *P. plantaginea* fixed on its proboscis. E, Pyralid moth visiting *P. stachyodes*. F, Pyralid moth with a pollinarium of *P. stachyodes* fixed on its proboscis.

visiting other flowers, bees carrying pollinaria brush them against the stigmatic surface, leaving pads of pollen, thus effecting pollination. Bees carrying one–four pollinaria were recorded. Syrphid flies (probably of genus *Salpingo-gaster*) were recorded at flowers but they did not dislodge pollinaria. Syrphid flies were recorded as co-pollinators of *P. densiflora* in Curitiba Paraná State, Southern Brazil) (Singer and Cocucci, 1999). In 2000, about 52 % of the flowers (966/1841) set fruits and individual fruiting success ranged from 18 to 90 %.

Prescottia plantaginea. Leaves are linear to slightly attenuate, light green in colour and often deteriorate somewhat during anthesis. Inflorescences bear up to 120 flowers which are coriaceous and light green in colour (Fig. 2C). Untouched flowers may keep their fresh appearance for 7–10 d. Just after dusk (approx. 1800 h), the flowers emit a sweet, spicy fragrance which, in cultivated plants, can be perceived until 2300–2400 h. Nocturnal scent emission, together with the inconspicuous flower colouration fit very well with the moth-pollination syndrome (van der Pijl and Dodson, 1966).

Flowers were visited and pollinated by Pyralidae moths (two morphospecies) (Fig. 2C and D). Pollinator activity was perceived from dusk (approx. 1800 h until approx. 1930 h). The moths often displayed a quite passive behaviour, remaining for 2–45 min on a given inflorescence. They often probed several flowers, but also perched on the inflorescences without exploring the flowers. The pollination mechanism is as follows: when the insects probe the flowers searching for nectar, they press the ventral surface of their proboscis against the viscidium, thus fixing the pollinarium. The pollinarium is removed when the moths leave the flowers. Pyralid moths were sighted carrying only one pollinarium. A pollinarium-carrying insect that visits another flower will brush the pollinarium (or pollinaria) against the broad stigmatic surface leaving pads of pollen, thus pollinating the flower. In several instances the moths failed to dislodge the pollinaria. It seems that on many occasions the moth's slender proboscis enters the flower at such an angle that viscidia are not disturbed. Unidentified Geometridae moths were frequently sighted at the flowers, but they never removed pollinaria. In 2000, about 30 % of the flowers (193/645) set fruits. Individual fruiting success ranged from 0 to 70 %.

Prescottia stachyodes. Leaves are erect, long-petiolated and lanceolate in shape, being light green, with a whitish median rib. Inflorescences are tall, up to 140 cm in length and may bear up to 180 flowers each. Flowers are protandrous (Figs 3 and 4A, B) and light green in colour (Fig. 2E). From flower opening and for about 3 d thereafter, the column is presented directed towards the lip (Figs 3 and 4A, B). At this stage, the stigmatic surface is inaccessible and the flower can only act as a pollen donor. After this stage, the column gradually acquires an opposite position, pointing towards the flower entrance (Figs 3 and 4C, D). Now, the stigmatic surface is completely exposed. At this stage, the viscidium is dry and the flower can only act as pollen receiver. Untouched flowers may keep their
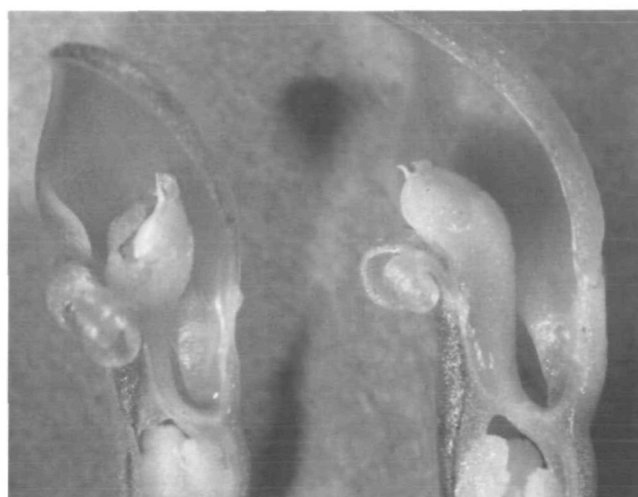

FIG. 3. Protandry in *Prescottia stachyodes*. The flower on the left is in the male phase, that on the right is in the female phase. Notice the different position of the column in the two flower phases.

fresh appearance for about 10–12 d and emit a strong, sweet and spicy fragrance from dusk, approx. 1800 h, and during the night (in cultivated plants until 2300–2400 h).

Two morphospecies of Pyralidae moths were recorded as pollinators (Fig. 2E and F). The period of pollinator activity was similar to that recorded for *P. plantaginea*, from 1800 to 2000 h. The moths visited inflorescences for 15–120 s, probing several flowers. Insects removed pollinaria when visiting the younger (male phase) (Fig. 4A and B) flowers, towards the inflorescence apex. Except for protandry, the pollination mechanism is much the same as that of *P. plantaginea*. Moths carrying pollinaria (only insects carrying one pollinarium were sighted) which visited the oldest (female phase) flowers at the base of the inflorescence brushed the pollinaria against the broad stigmatic surface, leaving pads of pollen and effecting pollination (Fig. 4C and D). In 2000, about 79 % (737/937) of the available flowers set fruits, and fruiting success per individual ranged from 52 to 98 %.

Breeding system

Results are summarized in Table 1. Plants are self-compatible but pollinator-dependent, i.e. pollinators are needed for the plants to set fruits. Only *P. densiflora* showed significant differences between self- and cross-pollination, favouring cross- over self-pollination (Table 1). This seems to suggest that inbreeding depression could occur in *P. densiflora*. However, larger samples, with more individuals and replicates, are necessary to confirm this. To our knowledge, there are no previous reports on the breeding systems of Prescottinae orchids. Since the plants are self-compatible and pollinators often visit several flowers of the same inflorescence, some of the fruit set may be the result of self-pollination in a broader sense (geitonogamy). Self-pollination in the strictest sense (pollen of the same flower deposited on its own stigmatic surface) may be a rare event, since the pollinarium is removed when the insect leaves the flower.
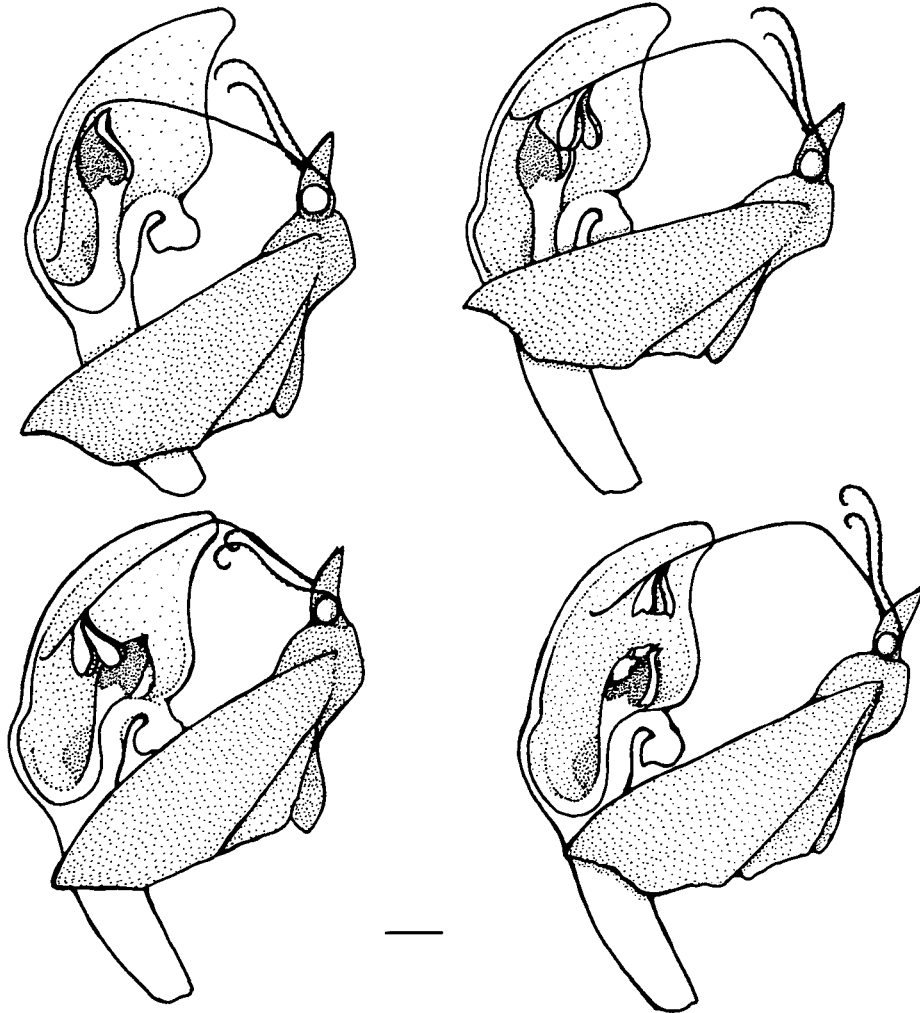
FIG. 4. Pollination mechanism in *P. stachyodes*. A, Pyralid moth probes a flower in male phase. B, The insect removes the pollinarium. C, A pollinarium-bearing moth probes a flower in the female phase. D, The stigmatic surface is brushed with the pollinarium and pads of pollen are deposited.

A combination of floral features may increase the chances of cross-pollination. First, the texture of the pollinarium, which is granular, allows clumps of pollen (instead of whole pollinia) to be left on the stigmatic surfaces. This means that several flowers can be pollinated by the pollen content of one pollinarium (Dressler, 1993; Singer and Cocucci, 1999). Second, the stigmatic surfaces are broad and slightly convex. This increases the chances of receiving multiple pollen loads (i.e either from the same inflorescence or a different one) and, consequently, of cross-pollination. In *P. stachyodes*, protandry clearly increases the chances of cross-pollination. Since the younger and uppermost flowers act as pollen donors, insects will fix pollinaria just before leaving the inflorescence, thus reducing the chances of self-pollination. Insects bearing pollinaria that visit another inflorescence tend to start their visits at the basal-most and older flowers of an inflorescence. These flowers may be in the female phase and will receive pollen pads on their exposed stigmatic surfaces, thus being pollinated. There is a chance that moths with fixed pollinaria will return to the basalmost (female phase) flowers of the same inflorescence,

thus promoting self-pollination (geitonogamy). During our observations, such behaviour was recorded only in *P. plantaginea*, which is non-protandrous. Protandry may occur in the rupicolous *P. montana* Barb. Rodr. This species is, according to our preliminary observations, self-compatible and pollinator-dependent. This species also shows flower traits suggesting moth-pollination, such as greenish, night-scented flowers.

Remarkably, *P. densiflora* and *P. stachyodes* displayed surprisingly high fruiting successes (52 and 79 %, respectively). In a recent review on orchid fruiting success, Neiland and Wilcock (1998) suggested that tropical orchids, either nectarless or nectariferous, display fruiting successes lower than 50 %. In our opinion, a combination of all the factors discussed above may account for the high fruit set.

Evolutionary ideas on the occurrence of protandry in the genus Prescottia

Protandry in terrestrial orchids has traditionally been associated with bumble-bee pollination (Catling, 1983, and

references therein), since bumble-bees tend to systematically visit inflorescences from the base (with flowers in female phase) towards the apex (with flowers in male phase). Given the evidence presented here, this idea needs to be reconsidered. Protandry is also known to occur in orchid genera of the subtribes Spiranthinae (Catling, 1983 and references therein; Catling and Catling, 1991) and Goodyerinae (Ackermann 1975, and references therein). According to Dressler (1993), the three orchid subtribes Goodyerinae, Prescottinae and Spiranthinae are phylogenetically related and were included in the tribe Cranichideae, which also includes subtribes Manniellinae, Pachyplectroninae and Cranichidinae (Dressler, 1993). The orchid subtribes Spiranthinae and Prescottinae are probably sister groups and share a number of features, such as the granular texture of their pollinaria and velamen type (Dressler, 1993). The genus *Prescottia* also shows the two retrorse lobules of the lip (probably nectaries) which are typical of subtribe Spiranthinae (Dressler, 1993). In both the subtribes Spiranthinae (Catling, 1983) and Goodyerinae (Ackermann, 1975) protandry involves column movements. At least in *Spiranthes* (Spiranthinae), column movements are effected through the enlargement of cells at the base of both the column and lip (Catling, 1983). After examining several preserved flowers of *P. densiflora* and *P. plantaginea*, we found no evidence of protandry (in the form of column displacement) for these two species. However, protandry not only involves column displacement, but also viscidium drying (Ackermann, 1975; Catling, 1983). It is possible that species lacking column displacement show viscidium drying at some stage of development. This subject needs further investigation.

Anatomical studies are needed to check if the protandry verified in *Prescottia stachyodes* is homologous to that previously recorded in subtribes Spiranthinae (Catling, 1983) and Goodyerinae (Ackermann, 1975). If so, this will provide further evidence of the relatedness of the three orchid subtribes. Further sampling would be worthwhile to assess how widespread protandry is, both in the genus *Prescottia* and in the subtribe Prescottinae as a whole. Only then can the taxonomic and phylogenetic value of this floral feature be fully understood. Obviously, further studies are needed, but it is tempting to suggest that protandry could have been present in a common ancestor or may have evolved early in the tribe Cranichideae, thus representing a primitive condition.

CONCLUSIONS

Moth-pollination is reported for the first time in the genus *Prescottia* and the subtribe Prescottinae. Previous reports indicate calliphorid flies and wasps as co-pollinators of

Myrosmodes cochleare (Berry and Calvo, 1991), and sweat bees (Halictidae) and syrphid flies as co-pollinators of *Prescottia densiflora* (Singer and Cocucci, 1999). This latter report is confirmed here. As far as we know, this is the first report of protandry in the genus *Prescottia* and in the subtribe Prescottinae.

ACKNOWLEDGEMENTS

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