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POLLINATION BIOLOGY OF *HABENARIA PARVIFLORA* (ORCHIDACEAE: HABENARIINAE) IN SOUTHEASTERN BRAZIL

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ABSTRACT: Singer, R. B. 2001. Pollination biology of *Habenaria parviflora* (Orchidaceae: Habenariinae) in southeastern Brazil. *Darwiniana* 39(3-4): 201-207.

The pollination biology of *Habenaria parviflora* (Orchidaceae: Habenariinae) was studied in Picinguaba, São Paulo State, southeastern Brazil in order to broaden our knowledge on the pollination biology and functional morphology of this terrestrial orchid genus. Plants are self-compatible but pollinator-dependent. The recorded pollinators were crane-fly (Diptera: Tipulidae) females and pyralid moths (Lepidoptera: Pyralidae). The pollination mechanism is the same regardless of the kind of pollinator: the pollinaria are fixed on the insect proboscis through the glove-like viscidium and removed when the insects leave the flowers. A pollinarium-carrying insect visiting another flower will brush the stigmatic surfaces and leave clumps of massulae, thus effecting pollination. This mode of pollinarium fixation is, as far as we know, reported for the first time for the genus *Habenaria* and is a clear consequence of viscidium's morphology. The involute, glove-like viscidium is a unique flower feature among neotropical *Habenaria* species. Flower features promoting cross-pollination are briefly discussed. This is also the first report of orchid pollination by crane-flies (Tipulidae) in the neotropics.

Key words: *Habenaria*, Orchidaceae, Orchids, Flower morphology, Moths, Pyralidae, Tipulidae, Crane-flies

RESUMEN: Singer, R. B. 2001. Biología de la polinización de *Habenaria parviflora* (Orchidaceae: Habenariinae) en el sudeste del Brasil. *Darwiniana* 39(3-4): 201-207.

Se estudió la biologia floral de *Habenaria parviflora* (Orchidaceae: Habenariinae) en Picinguaba, Estado de São Paulo, sudeste del Brasil, con la finalidad de incrementar el conocimiento sobre la biología de la polinización y morfología funcional de este género de Orquídeas terrestres. Estas plantas son autocompatibles, pero polinizador-dependientes. Los polinizadores registrados fueron hembras de Tipúlidos (Diptera: Tipulidae) y polillas Pyralidae (Lepidoptera: Pyralidae). El mecanismo de polinización es el mismo, independientemente del tipo de polinizador: los polinarios se fijan a la probóscide de los polinizadores a través de su viscidio en forma de guante, y son removidos cuando los insectos dejan las flores. Cuando visita otra flor, un insecto que lleva polinários los rozará contra las superfícies estigmáticas, dejando acúmulos de másulas, efectuando así la polinización. Hasta donde se sabe, este tipo de fijación de polinarios es informado por primera vez para el género *Habenaria* y es una clara consecuencia de la morfología del viscidio. El viscidio involuto, en forma de guante, es una característica única entre las especies neotropicales del género *Habenaria*. Los caracteres florales que favorecen la polinización cruzada son discutidos brevemente. Éste es también hasta donde sabemos, el primer registro de polinización de orquídeas por Tipúlidos (Tipulidae) en los neotrópicos.

Palabras clave: *Habenaria*, Orchidaceae, Orquídeas, Pollillas, Pyralidae, Tipulidae, Morfología floral.

INTRODUCTION

The cosmopolitan orchid genus *Habenaria* Willd. contains about 600 species, 170 of which have been referred to the Brazilian orchid flora (Hoehne, 1940). *Habenaria parviflora* Lindl. has a noteworthy wide distribution, occurring from central Argentina to southeastern Brazil (Cocucci, 1954; Correa, 1996; Singer & Cocucci, 1997).

Most pollination reports for *Habenaria* involve different kinds of moths or butterflies (Lepidoptera) (Dressler, 1993; Singer & Cocucci, 1997), althought *H. obtusata* is reportedly pollinated by mosquitoes (Culicidae) and moths as well (Stoutamire, 1968; Thien, 1969; Voss & Riefner, 1983). In a recent report of flower morphology and pollination biology of some *Habenaria* species occurring in central Argentina, Singer & Cocucci (1997) suggested that *Habenaria parviflora* (as *H. montevidensis*) should be pollinated by both, mosquitoes and moths. Owing to its particular viscidium shape, it was suggested (Singer & Cocucci, 1997) that the pollinaria of *H. parviflora* should be fixed on the proboscis of pollinators and not on the eyes like in other studied South American species. During our studies on the reproductive biology of southern Brazilian orchids, we had the opportunity of testing both ideas. The aims of the present contribution are: 1) to broaden our knowledge about the pollination biology of South American *Habenaria* and 2) to highlight the functional morphology of this noteworthy orchid species.

METHODS

Studies were conducted in Picinguaba (Parque Estadual Serra do Mar, Ubatuba, São Paulo, ca. 23° 15´, 23° 55´S and 44° 45´, 44° 55´W; altitude at a study site ca. 30 m.a.s.l.). *Habenaria parviflora* occurs in natural or man-made grasslands. The studied population occurs in the neighbourhood of the Park research base. The study site is surrounded by Atlantic Rain Forest ("Mata Atlântica") vegetation. The annual rainfall is about 2600 mm and the annual average temperature is about 22 °C. Observations were made through 17 field visits, from October 18-25, 1998, September 28 to October 10, 1999 and October 25-31, 2000. As a whole, about 35 hours and 25 minutes were spent in observations. The observation period ranged from 19 to 24 hours, in agreement with scent production and pollinator's activity at flowers. In 1998, 24 plants (totalling about 570 flowers) were available. Only six plants (totalling about 135 flowers) were available in 1999 and 14 plants (totalling about 520 flowers) in 2000. Flower morphology was studied using flowers preserved in ethanol 70 %. Plant vouchers are deposited at UEC (voucher number: *Habenaria parviflora*, R. B. Singer 98/ 101). Drawings were made using a stereomicroscope with a camera lucida attachment. In 2000, the breeding system was studied with six bagged, inflorescence-bearing plants totalling 277 flowers. Five flowers of each inflorescence were emasculated and the same number of flowers per inflorescence were self and cross-pollinated. A total of 187 remaining flowers were left untreated and were considered controls. All treatments were performed in each inflorescence. Given the small size of flower parts, pollinaria were removed with the help of entomological ("000" size) pins. Insect behaviour at flowers was recorded through field notes and photographs. Insect behaviour was traced with the help of a flashlight; a device which apparently did not disturb normal 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 taxonomic and morphological concepts of Dressler (1993) are followed.

RESULTS AND DISCUSSION

Plant features: owing to a combination of vegetative and floral features (well-developed leaves along the stem, plant size and inconspicuous flowers), *Habenaria parviflora* was included in an artificial and heterogeneous grouping called "the Leptoceras group" by Hoehne (1940). In fact, plants of *H. parviflora* are quite variable in size, reaching 15-100 cm in length. Plants occurring in well-exposed areas tend to be smaller and yellowish in colour. Plants surrounded by grasses tend to be higher and greener. Inflorescences are terminal spikes bearing up to 60 flowers. Flowers are resupinate and green or yellowish-green and bear a noticeable spur (Fig. 1 A) about 6.6 mm in length (Singer & Cocucci 1997). Readers interested in the size and shape of the perianth parts are referred to Hoehne (1940) and Cocucci (1954). The sepals are sturdier and fleshier than the petals and the lateral petals are partially held by the concave dorsal sepal. The dorsal sepal plus the lateral petals build a hood-like structure, which partially hides the column. The lip is distinctly trilobed and posteriorly prolonged in the spur (Fig. 1A) (Singer & Cocucci 1997). Nectar is clearly noticed at the bottom of the spur. Nectar secretion and nectary structure are beyond the scope of this contribution, see Galetto et al. (1997) for fine details on the nectary structure and nectar secretion in some related *Habenaria* species. The rostellum is trilobed, the two lateral lobes being inconspicuous and each one presenting a viscidium at its end. The median rostellar lobe is tooth-like (Fig. 1C) and is placed behind the spur entrance. Two small auricles (Fig. 1 D), one at each side of the column, are hidden by the lateral petals (Singer &

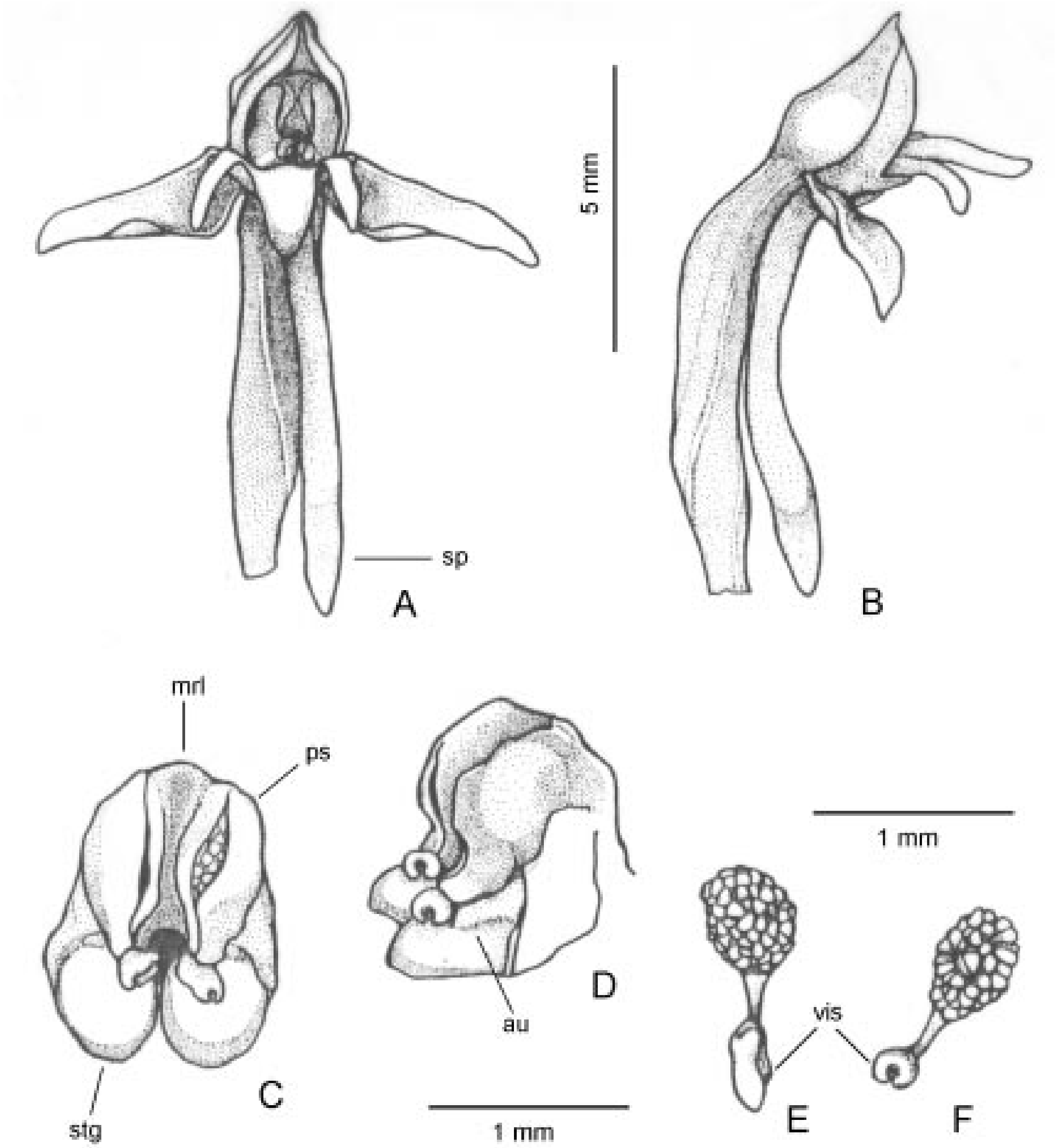

Fig. 1.- *Habenaria parviflora.* Flower features: A-B: flower. A: front view. B: lateral view. C-D: column. C: frontal view. D: lateral view. E-F: pollinaria. E: frontal view. F: lateral view. au: auricle. mrl: median rostellar lobe. ps: pollen sac. sp: spur. stg: stigmatic surface. vis: viscidium.

Cocucci 1997). The anther is erect and holds two pollinaria (until Dressler 1993 such structures were referred as “hemipollinaria”). Each pollinarium consists of a pollinium made up by ca. 70 massulae (Singer & Cocucci 1997), a hard, hyaline caudicle and a terminal, involute, glove-like viscidium (Fig. 1 E-F). The involute viscidium is, as far as we know, a unique and distinctive feature among South American *Habenaria* species (Hoehne 1940). Most *Habenaria* flowers show pad-like subcircular, slightly convex viscidia (Cocucci 1954, Hoehne 1940, Singer & Cocucci 1997) which usually attach to the surface of pollinator's eyes (Singer & Cocucci ,1997; Stoutamire, 1968; Thien, 1969; Voss & Riefner, 1983). There are two short, convex stigmatic surfaces (Fig. 1 C) just below the pollen sacs, surrounding the spur entrance.

Just after dusk (ca. 18 hours) the flowers emit a sweet, spicy fragrance which can be perceived up to 23-24 hours. Nocturnal scent production, together with the inconspicuous flower coloration fit very well the moth-pollination syndrome (Van der Pijl & Dodson, 1966).

Pollination biology: flowers were visited and pollinated by females of two mosquito-like, unidentified species of Tipulidae (Diptera) (probably of the genus *Leptotarsus* Guérin-Meneville) (Fig. 2 A-B) and several species of Pyralidae moths (Fig. 2 C-D). Insects often displayed a quite passive behaviour, staying for 2-45 minutes on a given inflorescence. Insects often probed several flowers, but also perched on the inflorescences without exploring the flowers. Crane-flies (Tipulidae) were always seen carrying and dislodging pollinaria. The crane-flies carried 1-5 pollinaria each. Pyralid moths were only sighted carrying one pollinarium each. The pollination mechanism is the same for both pollinators: when an insect probes the flowers searching for nectar, its proboscis is involved by the glove-like viscidium and the pollinarium is therefore removed. To fix the pollinarium, pressure has to be laterally exerted against the viscidium. This, together with the viscidium shape prevents that both pollinaria get fixed at the same time. In order to remove both pollinaria, insects have to probe the flowers more than once. However, as said above, pyralid moths were sighted carrying only one pollinarium. A pollinarium-carrying insect, which visits another flower, will brush the pollinarium (or pollinaria) against the bulky stigmatic surfaces depositing clumps of massulae and pollinating the flower. In several instances the moths failed to dislodge pollinaria. It seems that in many occasions the slender moth proboscis enters the spur at such an angle that viscidia are not disturbed. The straight and rigid proboscis of the crane-flies (Tipulidae) seems better suited for pollinarium removal. Crane-flies are rarely recorded as plant pollinators, but many genera show long proboscides which suggest that these insects may probe flowers for nectar (Grimaldi, 1999). There are a few records of Tipulidae pollinating European orchids of the genera *Listera* (Listerinae) and *Coeloglossum* (Orchidinae) (Van der Pijl & Dodson, 1966). We have occasionally observed crane-flies (apparently the same morphospecies which visit *H. parviflora*) visiting flowers of *Prescottia plantaginea* Lindl. (Orchidaceae: Prescottinae), thought they apparently did not remove pollinaria. Unidentified Geometridae moths were frequently sighted at flowers of *H. parviflora*, but in any event these moths removed pollinaria. Moth pollination has recently been confirmed for some *Habenaria* spp. in central Argentina (Singer & Cocucci, 1997). *Habenaria hexaptera* (as *H. hyeronimii*) is pollinated by the noctuid moth *Rachiplusia nu* (Noctuidae) and *Habenaria gourlieana* is pollinated by moths of *Agrius cingulatus* and *Manduca sexta* (both, Sphingidae) (Singer & Cocucci 1997). Indirect evidence of moth-pollination, in the form of moth scales on the stigmatic surfaces was presented for *H. rupicola* and *H. pauciflora* (this last species from Chile) (Singer & Cocucci, 1997). Mosquito pollination has been reported for the boreal *H. obtusata* (Stoutamire, 1969; Thien, 1968). These reports involved "true" mosquitoes of genus *Aedes* (Culicidae) and Geometridae moths of genus *Xanthorroe* as well (Thien, 1968; Stoutamire, 1969). Noteworthy, Voss & Riefner (1983) more recently reported that moths of genus *Anageshna* (Pyralidae) also pollinate *H. obtusata*. In all the aforementioned reports, the pollinaria were reported to be fixed on the insect eyes. *Habenaria decaryana* from Madagascar does also attach its pollinaria in this way (Nilsson & Jonsson, 1985). However, some African *Habenaria* species have been reported to fix their pollinaria on the venter (sternotriby) or in the forelegs (Knuth & al., 1905, Vogel, 1954) of their moth pollinators. Pollinarium fixation on the proboscis is, as far as we know, reported for the first time for *Habenaria.*

Reproductive success (number of fruits over total flowers) could not be calculated, since unbagged inflorescences were cut by the Park inhabitants (the study area is locally used as a car-parking area). In Central Argentina, a population of *H. parviflora* (reported as *H. montevidensis*) had 61 % of its flowers pollinated (Singer & Cocucci, 1997). The same population had a male efficiency factor (the ratio between the percentage of pollinated flowers over the percentage of flowers acting as pollen

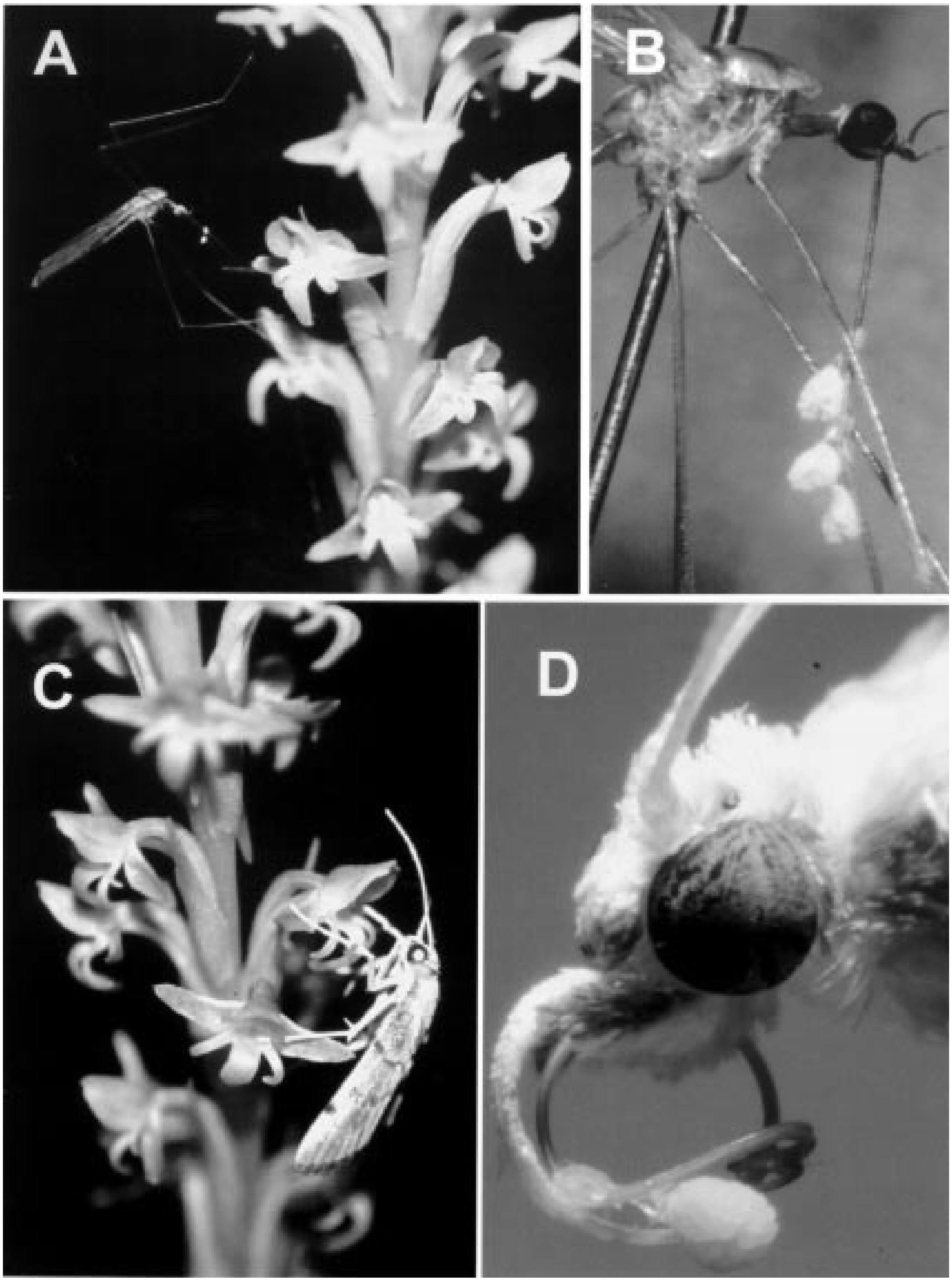

Fig. 2.- The flowers and their pollinators. A-B: Tipulidae (Diptera) females. A: visiting an inflorescence with a pollinarium attached to its proboscis. B: specimen with three pollinaria attached to its proboscis. C-D: Pyralid moths. C: specimen visiting an inflorescence. D: individual with a pollinarium attached to its proboscis.

donors) of 0.6 (Singer & Cocucci , 1997). This means that 100 pollinaria were removed to pollinate 60 flowers, or, in other words, that more pollinaria were removed than flowers were pollinated. The fixed pollinaria may in some way disturb the insects since Tipulidae females were often seen trying to clean the pollinaria from their proboscides with the forelegs.

Remarkably, Neiland & Wilcock (1998) indicated that tropical orchids (either nectarless or nectariferous) display low fruit productivities (less than 50 %). The data obtained for the Argentinian *H. parviflora* population (Singer & Cocucci, 1997) apparently contradict Neiland & Wilcox (1998) statement.

Breeding system: Plants are self-compatible but pollinator-dependent. This means that pollinators are needed for the plants to set fruits. Neither control (untreated) flowers (0 fruits/ 187 flowers) nor the emasculated ones (0 fruits/ 30 flowers) set fruits. Either self-pollinated or cross-pollinated flowers displayed very high and similar fruit sets, with 93.3 % (28/30) and 96.7 % (29/30) of fruiting success, respectively. Self-compatibility seems to be quite widespread among Orchidaceae (Dressler, 1981; Van der Pijl & Dodson, 1966). Since the plants are self-compatible and the pollinators are often quite passive, frequently staying several minutes and visiting several flowers of the same inflorescence, part of the fruit set may be due to geitonogamous self-pollination. Self-pollination in the strictest sense (pollen of the same flower deposited in its own stigmas) may be a rare event since the pollinarium is removed when the insect leaves the flower. Still, a combination of flower features may increase the chances of cross-pollination: the texture of the pollinarium, which is made up by several subunits (“massulae”) and the fact that clumps of massulae (instead of whole pollinia) are left on the stigmatic surfaces. This means that several flowers can be pollinated with the pollen from one pollinarium (Singer & Cocucci, 1997). In addition, the stigmatic surfaces are broad and convex which increases the chances of receiving mixed pollen loads (of the same and other flowers) and, consequently of cross-pollination. Finally, the sectile condition of the pollinaria per se guarantees a considerable degree of genetic diversity (Freudenstein & Rassmusen, 1997). According to Freudenstein & Rassmusen (1997) each massula derives from a single pollen mother cell. So, each massula represents a different genotype. Since massulae are left in clumps and a single stigmatic surface could receive many pollen loads, the chances of multi-pollen genotype contributions to a single capsule are increased (Freudenstein & Rassmusen, 1997).

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