Pollination, Flower Longevity, and Reproductive Biology of *Gongora quinquenervis* Ruíz and Pavón (Orchidaceae) in an Atlantic Forest Fragment of Pernambuco, Brazil

P. Martini[1], C. Schlindwein[1], and A. Montenegro[2]

[1] Universidade Federal de Pernambuco, Centro de Ciências Biológicas, Departamento de Botânica, Pernambuco, Brazil
[2] Universidade Federal Rural de Pernambuco, Departamento de Biologia, Área de Zoologia, Pernambuco, Brazil

Received: August 4, 2003; Accepted: August 21, 2003

Abstract: The pollinators, flower longevity, and reproductive success of *Gongora quinquenervis* were studied in Refúgio Ecológico Charles Darwin, a preserved fragment of Atlantic Forest in Pernambuco. *G. quinquenervis* is epiphytic, and its flowers have osmophores, glands that produce aromatic volatiles, that are collected by males of Euglossini (Hymenoptera, Apidae). Every flower of an inflorescence opened simultaneously, however, overlaps in floral phases between individuals were low. Pollinaria deposition on the stigma caused immediate wilting of the perianth, while pollinaria removal had no influence on flower longevity. The reproductive system experiments showed that the species is self-compatible. The characteristics of floral morphology and its highly specialized pollination mechanism are efficient in limiting autogamy and geitonogamy and favouring cross-pollination. Three species of *Euglossa* were found (*E. cordata, E. perpulchra* and an undescribed species) visiting the flowers of *G. quinquenervis*. All these efficiently removed the pollinaria of the flowers, which adhered to the posterior margin of the scutellum. Even though there was a high pollinaria removal rate by pollinators, the reproductive success in the field was extraordinarily low. We suggest that low fruit set, despite high pollinator frequency, is a result of a combination of the particular phonological characteristics of *G. quinquenervis*, such as short flower longevity and low overlap of flowering phases between individuals, leading to the reduced population of this orchid in the degraded Atlantic Forest. Conservation measures are necessary to guarantee the survival of *G. quinquenervis* in the northern part of the Atlantic Forest of Brazil.

Key words: Pollination, Euglossini, *Gongora quinquenervis*, reproductive success, floral longevity.

Introduction

The relationship between orchids and the males of Euglossini (Apidae) has been the object of various studies during recent decades because of its high degree of complexity, which is reflected in the flower structures of many orchids, in the absence of nectar and in the morphology and behaviour of those bees which clearly differentiates them from other Apidae (Rebêlo, 2001). The perfume of orchids, volatile lipids produced in floral glands (osmophores), not only functions in attracting male euglossine bees, but is also an actively exploited floral reward that is stored in the specialized hind tibiae of such bees (Dressler, 1982). Even though the exact function of these perfumes on the biology of these bees has not been elucidated, their production is a key factor in the evolution of many orchids (Crüger, 1865; Allen, 1950; Vogel, 1963; Dodson, 1962a, 1965; Dressler, 1967, 1982, 1993).

The period in which a flower is attractive and available to its pollinator can last from minutes to months (Van der Pijl and Dodson, 1966). Even though flower longevity may influence pollinator visitation or level of inbreeding, flower longevity is not often incorporated in studies of reproductive strategies of plants (Stratton, 1989; Ashman and Schoen, 1994). There are two factors that determine senescence: a physiological process independent of removal and deposition of pollen (Proctor and Harder, 1995), and one induced by pollinators, and determined by pollen deposition (Ackerman, 1989a) or removal (Devlin and Stephenson, 1984).

Gongora is an exclusively neotropical genus with approximately 55 species (Jenny, 1993). It is one of the 22 genera of the Stanhopeinae subtribe, which is exclusively euglossophilous, as is Catasetinae, and presents some of the most complex pollination mechanisms (Dressler, 1993). The taxonomy of *Gongora* is quite difficult and therefore further investigations are required to better distinguish this group of such complex flowers. *Gongora quinquenervis* is epiphytic and occurs in the Zona da Mata, Brejos de Altitude, and Matas Serranas of Pernambuco (Felix and Carvalho, 2002), and is also found in the Coastal Atlantic Forest. Quite a few natural history studies are known for Central America, describing observations on visitors to *Gongora* flowers (Allen, 1950; Dodson and Frymire, 1961; Dodson, 1962a, 1966, 1967; Van der Pijl and Dodson, 1966; Dressler, 1967). These works, however, give little emphasis to aspects of phenology, reproductive biology and pollination ecology. In Brazil, there are few works on floral and reproductive biology, or pollination ecology of euglossophilous orchids. Ruschi (1986) made observations focusing on the natural history of floral visitors of *Catasetum turidum, Coryanthes speciosa* and *Gongora bufonia* in the State of Espírito Santo. In the State of Pernambuco, Carvalho and Machado (2002) studied the floral biology of *Catasetum macrocarpum*. In this study we ask:

Plant biol. 5 (2003): 495 – 503

ISSN 1435-8603

1) Which bees pollinate *Gongora quinquenervis* at the study site? 2) What is the reproductive system of this species? 3) What is its flower longevity? 4) What is the influence of pollination and pollinaria removal on flower longevity? and 5) What is the species' reproductive success in the study site?

Materials and Methods

The field study was conducted at the Refúgio Ecológico Charles Darwin, a preserved Atlantic Forest remnant, located in an intensely fragmented forest area of the sugar-cane growth area of Northern Pernambuco (7°48′45″S and 34°57′12″W). The forest fragment studied consists of a regenerating forest. The field study period was from October 2000 through February 2001, and from October 2001 through March 2002. Considering the identity problems that are still found within *Gongora*, we use *Gongora quinquenervis* here in a very broad sense. We believe that careful work is still needed to sort out the species in this complex. A group of five individuals (control) of *Gongora quinquenervis* was observed during both flowering seasons.

Observations of vegetative morphology, flower morphology and flower colours were made. Scent production was measured by direct olfactory tests. Anthesis was described in detail by the observation of two non-manipulated inflorescences (n = 13 and n = 11 flowers), marked individually, where the time, duration and sequence of anthesis was recorded. The number of open flowers per inflorescence/day was recorded in the field (control) during both flowering periods. Stigma receptivity was measured in 15 non-manipulated and in 15 emasculated flowers using H_2O_2 (Galen and Plowright, 1987). Stigma receptivity was also verified through manual cross pollinations made hourly until fruit set could be observed on one of the tested flowers (n = 48 flowers). The areas of scent emission were detected using the neutral red test (Vogel, 1962). Flower longevity, from pre-anthesis to complete senescence, was observed in controls and in greenhouse-grown individuals. The greenhouse was located in the forest reserve. In this last case, three treatments were conducted: untouched flowers (plant 1), flowers emasculated at anthesis (plant 2) and flowers manually cross-pollinated at start of stigma receptivity (plant 3). Each treatment was composed of n = 15 flowers per plant. Flower longevity of controls exposed to flower visitors was registered. The possible influence of pollinaria removal and deposition in the stigmas over flower attractivity (by the interruption of scent production) was tested, comparing the frequency of visitations in 15 emasculated (plant 2) and 15 pollinated flowers (plant 3).

Flowering and fruiting phenology was followed weekly in the control group, growing on trees at distances varying from 5 to 8 m. The time an inflorescence takes until it reaches anthesis, as well as the time needed until a fruit capsule ripens, was recorded. The flowering pattern was determined following Newstron et al. (1994).

The reproductive system was studied using the methods of Radford et al. (1974). The treatments included spontaneous self-pollination tests (plant 1: n = 65 flowers), manual self-pollination (plant 4: n = 77 flowers), manual cross-pollination (plant 5: n = 66 flowers), apomixis (plant 2: n = 63 flowers) and pollination under natural conditions (5 controls: n = 563 flowers). The experiments were conducted in an insect-protected greenhouse located in the vicinity of the study site. For the manual pollination tests, the pollinaria were placed in the stigmas at the beginning of the female phase. The reproductive success was evaluated by counting fruits formed.

A seed viability test was done using fruits produced by manual cross-pollination and by manual self-pollination. The seed sowing technique used was that of Martini et al. (2001). Each treatment consisted of ten repetitions of 50 randomly picked seed samples, making a total of 500 seeds per treatment. Following sterilization, the samples were separated under a stereomicroscope placing a Petri dish over a millimetric grid. These were put to germinate in Petri dishes containing 10 ml of seed sowing media. The two tests were compared using Student's *t*-test (BioEstat 2.0).

The percentage of pollinaria deposition and removal was determined in the control group (n = 563 flowers) in both flowering seasons. This was determined by observing each flower of an inflorescence (n = 32 inflorescences) at the end of anthesis, with the purpose of verifying the effectiveness of the visitors.

The behaviour of the visitors was studied by direct observation from 6:30 to 16:30 and documented with photographs. Nine flower visitors were captured and marked individually on the mesoscutum with non-toxic paints as soon as they arrived on the flowers. The time each of these bees stayed on individual flowers was recorded. Some visitors were collected for later identification. The bees were deposited at the Entomological collection of the Plant Ecology Laboratory at the Universidade Federal de Pernambuco, and vouchers of *Gongora quinquenervis* in the Herbarium Geraldo Mariz, UFP/Universidade Federal de Pernambuco.

Results

Floral and vegetative morphology: *Gongora quinquenervis* is an epiphytic species with ovoid–oblong pseudobulbs, deeply grooved and ridged 2.5 – 7.0 cm in diameter and 7.5 – 10.0 cm long, and two elliptic–obovate leaves at the apex, plicate, suberect 25 – 37 cm long, 5 – 9 cm in diameter, margins undulate, short pseudopetiole. The inflorescence are pendant racemes 40 – 70 cm long, emerging from the base of the pseudobulb, 10 – 26 flowered and spirally distributed. Flowers are fragrant, yellow but strongly spotted with brownish-red and maroon. Dorsal sepal is erect, 2.0 cm long and 0.3 cm wide, apex and margins are lightly curved back. Lateral sepals are 2.7 cm long and 0.5 cm wide, reflexed with margins curved back. Petals are joined to sides of the column in the basal half, lanceolate, 0.8 cm long and 0.2 cm wide. Lip is fleshy, 3-lobed, side lobes each bearing a basal erect protuberance 2.0 cm long and 0.5 cm wide. See page 27 of Jenny (1993) for a descriptive drawing of a *Gongora* flower. The areas with the highest concentration of osmophores are the adaxial face of the lip at its base (hypochile) and lateral sepals. Ovary is 3.7 – 3.8 cm long, strongly arched downward from its median half. Pedicel is 1.3 – 1.4 cm long, bearing a lanceolate bract 0.4 cm wide. Column is arcuate, dilated towards the apex, 1.9 cm long and 0.2 cm wide, presenting the stigma in a small slit, the well developed stigma lobe is perfectly isolated from the anther. Anther is 0.4 cm long, 0.2 cm wide, apically placed on the column, bearing two hard clavate pollinia with protuberant viscidium, placed between anther and rostellum.

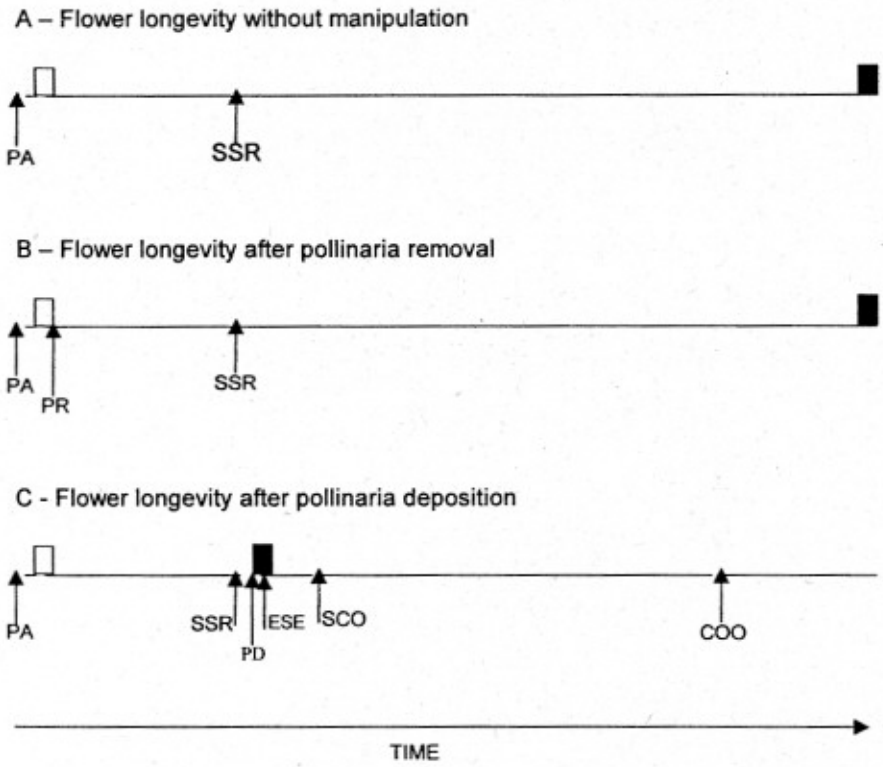

Fig. 1 Scheme of events throughout anthesis for *Gongora quinquenervis* in three treatments. (**A**) Unmanipulated flowers. (**B**) Flowers with pollinaria removed at the start of anthesis. (**C**) Pollinaria deposition at the beginning of the female phase. Pollination causes an immediate response such as end of scent emission, wilting of the perianth and change in orientation of floral parts. PA: pre-anthesis; □: start of anthesis; SSR: start of stigma receptivity; PD: pollinaria deposition; ESE: end of scent emission; ■: senescence; PR: pollinaria removal; SCO: swelling of column and ovary; COO: change in ovary orientation.

Anthesis

An inflorescence is formed within 32–38 days. Flowers started opening between 3:30–4:30 a.m. and were fully open by 6:30–7:30 a.m., which was considered the start of anthesis. This occurred earlier in those individuals growing in more light-exposed areas. Anthesis was simultaneous in all marked inflorescences and all flowers opened at the same time. The fragrance could be sensed at the start of anthesis and terminated with flower fading.

G. quinquenervis is a protandrous species. Visitors can remove the pollinaria at the beginning of anthesis. The hydrogen peroxide tests showed that stigmatic receptivity began between 23–24 h after the beginning of anthesis (n = 15) and remained until its end. On flowers that had their pollinaria removed at the beginning of anthesis, the start of the female phase did not differ from the non-manipulated flowers (n = 15).

Flower longevity

Anthesis lasted 4 days on non-manipulated and non-visited flowers. Senescence started at the end of the morning on the fifth day. When intensely visited or when pollinated, the flowers lasted only 2 days (n = 30). Intense flower visitation by dozens of male bees was necessary to trigger senescence in the control group. In this case, flowers frequently lasted only 3 days. Pollinaria removal had no influence on longevity when compared to the control group. Deposition on the stigma, however, shortened longevity by half (50 h) (Fig. **1**).

Attractivity was compared among non-manipulated and emasculated samples, and a reduction of attractivity was not noticed in the latter case. Meanwhile, after pollinaria deposition on the stigma, scent production ceased in 10 min (n = 28). Bees no longer visited these flowers. Flowers started fading approximately 1 h after pollination. Eight hours later the column started to swell and change its colour to green but kept the intense pattern of brownish-red and maroon spots. The column's arched down position straightened around 48 h after pollination, giving the faded flower a resupinate position. At this moment, the stigmatic slit became narrower until it closed completely.

Phenology

G. quinquenervis' flowering season started in early October and continued through early March. The floral pattern is annual, with most flowers open in December. Flowering is not continuous, each plant had 1–5 floral phases of a few days (Fig. **2**). The interval between floral phases varied from 1 day to 8 weeks (mean = 22 days; pd. = 7.3). The group presented a defined flowering period but showed no synchronisms, thus presenting little overlap of open flowers among individuals.

The development of new inflorescences was not inhibited on those manually-pollinated plants. New inflorescences developed even in the presence of older inflorescences with developing capsules. However, fruiting retards the development of new pseudobulbs. A fruit takes 56–60 days to develop, from pollination to dehiscence.

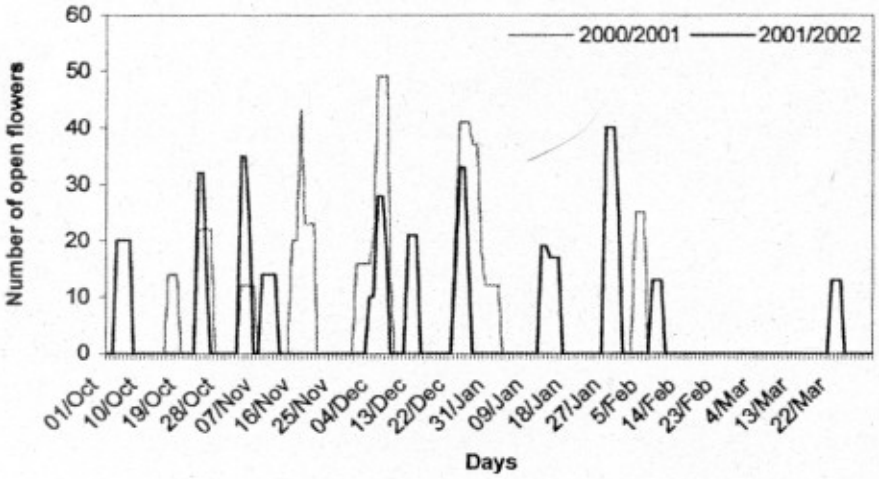

Fig. 2 Dynamics of *Gongora quinquenervis* flowering during the experiment. Number of open flowers on five individuals of *G. quinquenervis* between October 2000 and March 2002. While flower opening is simultaneous in all flowers of an inflorescence, inflorescence development and the intervals between floral phases are irregular.

Reproductive system

The controlled pollination experiments showed *G. quinquenervis* to be self-compatible, though 73% of manually cross-pollinated flowers produced fruits, while only 35% of manually self-pollinated flowers produced fruits (Table **1**). Fruit production of the controls was absent in 2000–2001 and 1.1% in 2001–2002. There was a higher *in vitro* germination rate among fruits produced by manual cross-pollination (98%) as compared to fruits produced by manual self-pollination (73%). The difference was statistically significant (Fig. **3**).

Table 1 Reproductive system of *Gongora quinquenervis*. The control flowers were exposed to visitors. The manual pollination tests were conducted inside a greenhouse located in the reserve, close to the control group (2001–2002)

Treatment	Number of flowers observed	Number of fruits produced	% of fruits produced
Control group (plants exposed to visitors)	285	3	1.1
Manual self-pollination	77	27	35
Spontaneous self-pollination	65	0	0
Apomixis	63	0	0
Manual cross-pollination	66	48	73

Flower visitors

The visitors to *Gongora quinquenervis* flowers were exclusively males of *Euglossa* (Euglossini) (Figs. **5A–D**). The most frequent visitor was *E. cordata*, being present throughout the whole flowering period. Thirty to 40 males of this species were continually present from early morning throughout the first hours of the afternoon. Males of *E. perpulchra*, a new species recorded for the first time during this study, were seen only during December and January, and visited flowers during the mornings only, with 5–6 individuals per inflorescence. Males of another undescribed dark blue *Euglossa* species were recorded as flower visitors only during February 2000. Males of *Eulaema flavescens* were observed during both flowering periods, hovering in front of the flowers but never landing. The visitation by *E. cordata* began at 7:00–7:30 and lasted until 15:30–16:00, with a peak recorded from 9:30 to 12:30, followed by a decline in intensity starting at 13:30. The amount of time spent by an *E. cordata* on an individual flower ranged from 18 s to 45 min (Fig. **4**). The same individual visited various flowers in the same inflorescence. The bees collected volatiles during most of the morning, returning about 2 h later in the afternoon. All three male of *Euglossa* species exploited the osmophores using the long hairs of their anterior tarsi, after which they hovered for approximately 5 s to transfer the volatiles to the storage area of the hind tibiae. This sequence was repeated during the collection of volatiles. A single flower could be exploited by four males at the same time, even from different species, without any aggressive behaviour among them. The flowers were generally much damaged by intense brushing of the surfaces to collect volatiles, especially the base of the lip (hypochile), which was frequently torn. While brushing the osmophores located on the median superior half of the lip, the males positioned themselves with their back facing down between the protuberances of the side lobes (Fig. **5A**). They held on to the lip with the help of the mid and posterior legs. The bees were then positioned to either pick up or remove the pollinaria when they fell, caused by a loss of motor coordination during volatile collection, which led them to fall backwards on the abaxial face of the column. The distance between the protuberances of the mesochile is similar to the width of the bee's dorsum. When sliding down the column the viscidium touches the posterior margins of the scutellum, placing the pollinaria onto the bee's back. In the three species studied the pollinarium was placed on the same area of their bodies (Figs. **5B–D**).

Bees with more than one pollinarium adhering to their scutellum were quite common. Exceptionally, pollinaria adhered to the mid-legs under the wings, which made flight difficult. The bees removed such misplaced pollinaria after landing.

After being removed by the bee, the pollinaria go through a dehydration process, which causes a bending of approximately 90° of the stipe. This process takes a few minutes. The pollinia also dry up. To be deposited on the stigma, these become positioned parallel to the bee's dorsum (Fig. **5D**). The action of the

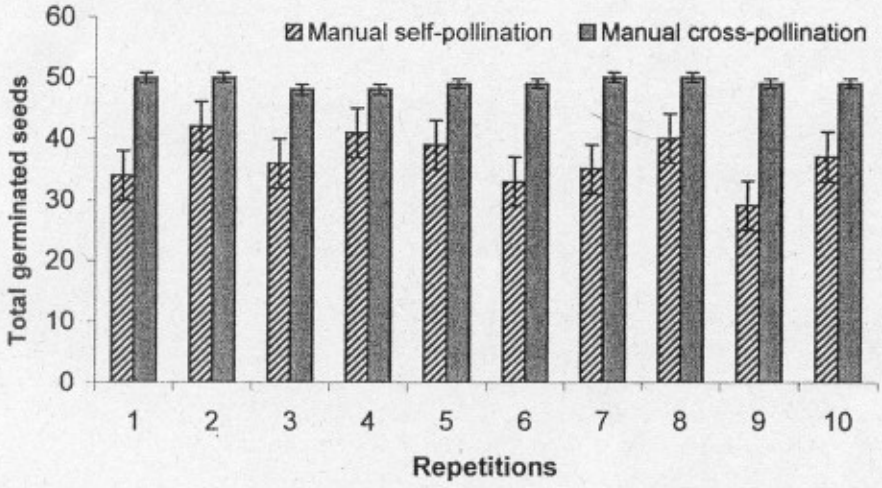

Fig. 3 *In vitro* growth of *Gongora quinquenervis* seeds produced by manual cross-pollination and manual self-pollination $x = 49.20 \pm 0.78$; $x = 36.6 \pm 4.03$ ($t = -9.69$; $gl = 18$; $p < 0.001$). Seed viability of cross-pollinated fruits is significant. Fifty seeds per sample were used in each treatment (50 × 10).

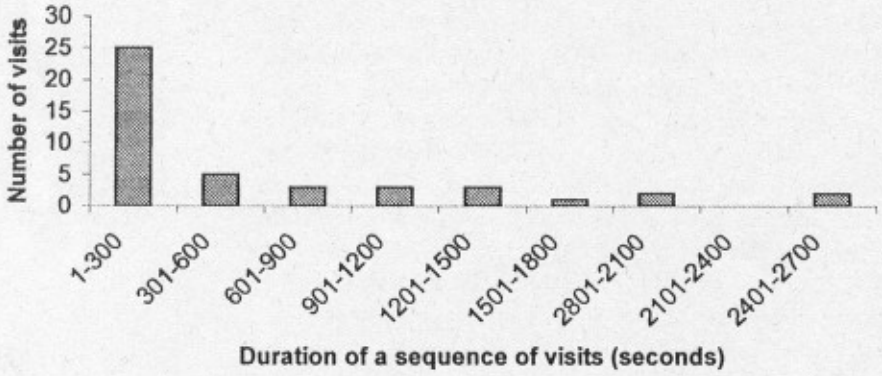

Fig. 4 Duration of *Euglossa cordata* visits in individual flowers of *Gongora quinquenervis*.

breeze on the flowers causes the release of the anther cap, which exposes the pollinarium that remains united to the column.

Removal and deposition of pollinaria

In both flowering seasons, there was a high rate of pollinaria removal, higher than 90% in 16 of the 32 observed inflorescences. 244 were removed in 2000–2001 from a total of 278 flowers (88%), and 249 were removed from a total of 285 flowers in 2001–2002 (87%) contrasting with the absence of female success (pollinaria deposition) in the first period, and 1.1% female success in the second. There was no deposition in the first period but three were deposited in the second. All formed fruits. No young individuals of *G. quinquenervis* were found in the vicinity of the study site.

Discussion

All flower visitors of *Gongora quinquenervis* in this study belong to the genus *Euglossa*. The observations made here agree with other authors concerning a close relationship between *G. quinquenervis* and males of *Euglossa:* Allen (1950), Dressler (1966) and Dodson (1962b). In this study, *E. cordata* was the most abundant visitor.

We believe *E. perpulchra* has a close relationship with *G. quinquenervis;* since pollinaria of other orchid species were not found attached to those bees, there is no evidence that this species visits other orchids. To our knowledge, the occurrence of *E. perpulchra* seems to be restricted to Northeastern Brazil. Besides Igarassu-Pernambuco, it was later captured in Camaragibe-Pernambuco (Martini, personal communication; Schlindwein, personal communication) and Serra Grande-Alagoas (Locatelli, personal communication). *Eulaema flavescens* was another species attracted by the flowers, however, without landing on them. The perfumes of euglossophilous flowers are generally highly specific, attracting one of only a few bee species (Dressler, 1967). This specificity could promote reproductive isolation, leading to the speciation of sympatric species, as in *Gongora aceras* and *Gongora napoensis* in the Andes (Gerlach and Schill, 1991). Williams and Whitten (1983) and Gerlach and Schill (1991) suggest that the species-specific scent composition contributes to the specificity of pollinators. One of the pollinators in this study *(E. cordata)* did not present high specificity since it was found with pollinaria of other orchid species. Considering the current lack of accessible information, the second most frequent pollinator *(E. perpulchra)* seemed to have more close relation with *G. quinquenervis.* In euglossine-pollinated orchids, it is known that the place of deposition of pollinaria on the bee's body is highly variable. This is considered to be a strategy to guarantee reproductive isolation (Dressler, 1982). The pollination mechanism in the

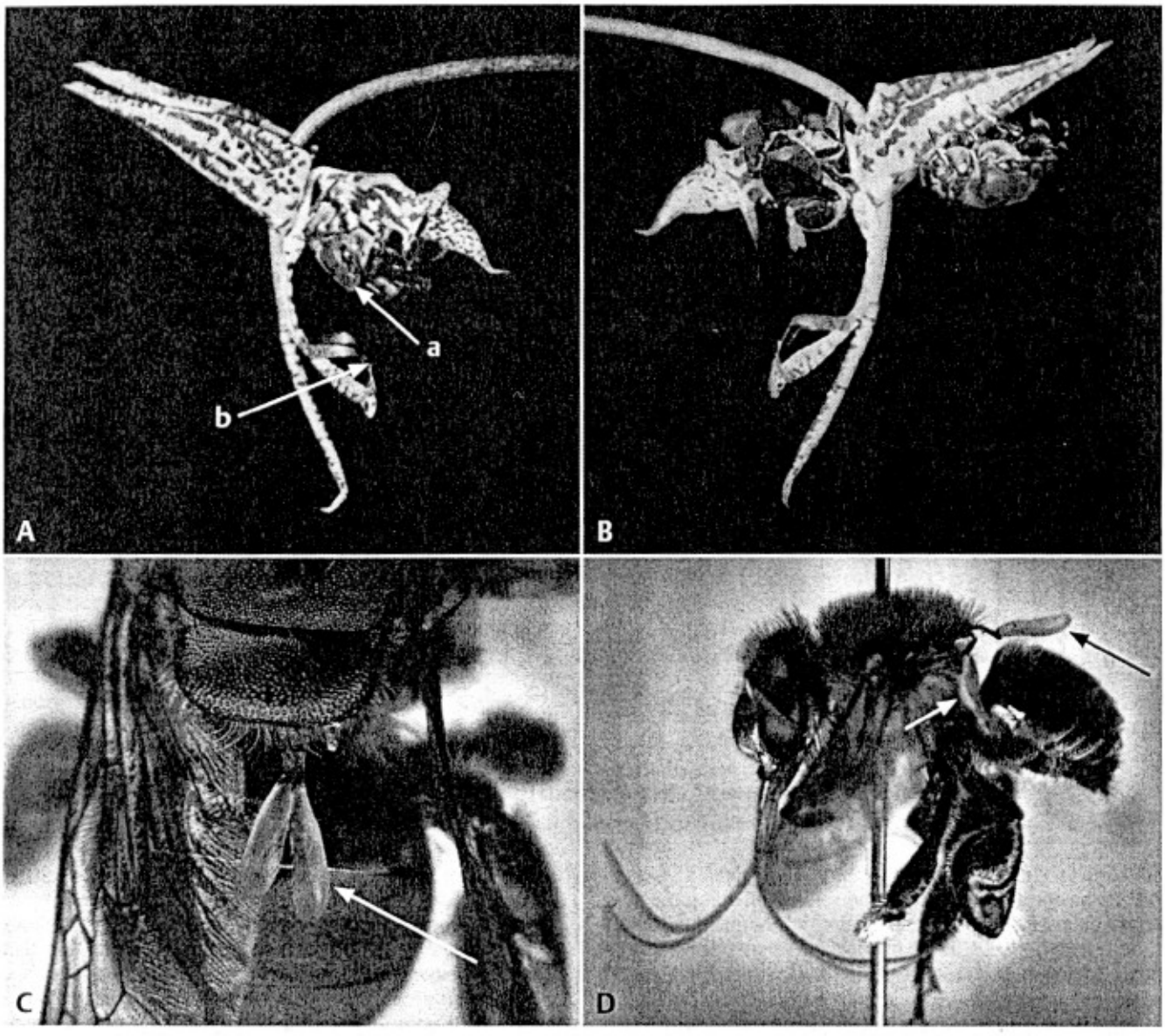

Fig. 5 (**A**) *Euglossa cordata* positioned on the lip moments before its fall, with the dorsum facing the inferior surface of column of *G. quinquenervis* (a) posterior margin of scutellum, (b) viscidium. (**B**) *Euglossa* sp. (left), with pollinaria attached to posterior margin of scutellum, and *E. cordata* (right) collecting scent. (**C**) *Euglossa cordata* with pollinaria attached to posterior margin of scutellum. (**D**) *E. perpulchra* with pollinaria attached to scutellum.

species studied here results in the deposition of pollinaria on the posterior margins of the scutellum, which was seen on all three *Euglossa* species found.

In the State of Pernambuco, as well as in other countries of Central and South America, there is some difficulty in identifying individuals of the genus *Gongora*. Studies on the pollination of other species/populations of *Gongora* in this State may reveal specific relations with other *Euglossa* species that could lead to the explanation of its reproductive isolation. *Gongora* is among the most highly evolved genera of the Epidendroideae, presenting a high degree of specificity and adaptations to pollinators, favouring reproductive isolation (Dressler, 1981; Feinsinger, 1983; Dressler, 1993).

Dodson and Frymire (1961) mention flower longevity of only 2 days for *Gongora maculata*. If this plant was intensely visited, the mechanical damage caused by the collection of volatiles might have caused its senescence, as observed in our study. We noticed that pollinarium deposition on the stigma triggers rapid wilting of the perianth, which could also explain the longevity of *G. maculata* observed by Dodson and Frymire (1961).

Pollinaria or pollinia removal on some orchid genera such as *Vanda, Arachnis, Aranda, Cattleya, Cymbidium, Dendrobium, Paphiopedilum, Phalaenopsis* and *Cymbidium* (Burg and Dijkman, 1967; Arditti et al., 1973; O'Neill, 1997) triggers a senescence process. In our experiments, the removal of pollinaria did not affect longevity; on the other hand, deposition caused imme-

diate wilting of the perianth. Miranda (1996) mentions that the genus *Gongora* has its flower longevity reduced by the removal of pollinaria, something that was not observed in our study with *G. quinquenervis*. The flowers of *G. quinquenervis* remained attractive to bees even when they had all their pollinaria removed on the first day of anthesis. The termination of scent emission in *Gongora* and *Catasetum* after pollinaria removal was mentioned by Arditti (1992). It happens with Catasetinae possibly because of the dioicy found among its species. The factor inducing early wilting in *G. quinquenervis* was the deposition of pollinaria on the stigma. The pollination quickly triggered a series of events such as end of scent emission, wilting, colour change, collapse of floral parts, and changes in orientation of the flowers. Such reactions are called post-pollination phenomena or post-pollination syndrome (Gori, 1983; Arditti, 1992; O'Neill, 1997). According to these authors, these phenomena are characterized by a series of events where a flower becomes specialized to ensure fertilization and nourishment of the developing embryo. This functional transition presents developmental processes associated with wilting of the perianth, pigmentation changes, ovary maturation, ovule differentiation, and female gametophyte development. The last three events do not occur in most tropical orchids during anthesis (Nazarov and Gerlach, 1997).

According to O'Neill (1997) and Weiss and Lamont (1997), wilting of the perianth and changes in floral organs serve as signals for insect pollinators to discriminate receptive flowers from those that have advanced to later stages of reproductive development. We noticed in our experiment that, a few minutes following pollinaria deposition on the stigmatic slit, the flower ceases scent emission, which was noticed by olfactory tests as well as by the termination of visitation. Approximately 1 h after pollination, the sepals, petals and lip started to fade and collapse, positioning themselves in a way to protect the stigma. We interpret such post-pollination changes as adaptations to protect the recently deposited pollinarium, avoiding its removal by later visitors. A similar phenomenon was described for *Bulbophyllum macranthum* (Millar, 1978) and *Cypripedium arientinum* (Case, 1964).

The stigmatic surface of *G. quinquenervis* is so small that it does not allow the two pollinia to be fully deposited inside. The stigma of this species does not present much adhesive on its surface, as found in many other orchids.

Reproductive system

G. quinquenervis did not show a self-incompatible mechanism; instead, it has a series of mechanisms to inhibit autogamy and geitonogamy: protandry, the isolation of pollinaria by the rostellum and anther cap, and dehydration of stipe and pollinia.

Protandry has been found in many species belonging to the Stanhopeinae (Dressler, 1993) and in the genus *Gongora*, such a characteristic has been constant in all species studied so far. Dodson and Frymire (1961) and Dodson (1962a) noticed that the flowers of *Gongora maculata* were effectively pollinated by *Euglossa viridissima* on the second day of anthesis, just a few hours before wilting, when the narrow stigmatic slit became more open and receptive, which was also observed in our study. Here, stigma receptivity took place one day after anthesis. According to Johnson and Nilsson (1999), in general, the selection of characters to prevent geitonogamy occurs when the pollinator is abundant, as in our study. In orchid flowers that tend to be revisted repeatedly by the same individual, such as in our study, protandry is considered to be effective (Johnson and Edwards, 2000). In Catasetinae, also exclusively euglossophilous, protandry occurs in some genera, but in this case, the stigma is receptive only after the pollinaria have been removed, as in *Mormodes* and *Dressleria* (Chase and Hills, 1992). This was not found for *G. quinquenervis*.

In many Orchidaceae, in order to be adequately positioned to penetrate the stigma of the next flower, pollinaria need to go through a dehydration process when removed by pollinators (Johnson and Edwards, 2000). According to Dressler (1968, 1981), in the case of euglossophily where bees tend to reenter the same flowers many times, this process decreases the risk of self-pollination, which places these genera among those that have most perfect mechanisms to prevent self-pollination and geitonogamy. A few minutes or even a few hours are necessary until the pollinaria are adequately placed to enter the stigma (Johnson and Edwards, 2000; Dodson, 1967). Dodson (1967) mentions this strategy in *Stanhopea* and *Gongora*. In *G. quinquenervis* pollinaria cannot be placed by the bee on the stigma immediately after removal, since the dehydration of pollinia and the bending of the stipe need to take place. Another characteristic that inhibits spontaneous self-pollination is the rostellar and anther morphology. The rostellum, a lobe originated by the stigma which separates it from the anther (Arditti, 1992; Dressler, 1993), is well developed in *G. quinquenervis*, forming along with the anther cap, an involucre that covers and isolates the pollinia from any contact with the stigmatic surface. The manual pollination experiments revealed that the species favours cross-pollination, instead of self-pollination, which produced 50% less fruits. Besides, the germination rates of manually cross-pollination produced seeds were significantly higher (98%) than that of those produced from self-pollination (73%).

Despite the capacity to produce fruits by self-pollination, some studies with orchids show a tendency to a reduction in the number of seeds (Peakall, 1989 *apud* Johnson and Edwards, 2000) and their fertility (Ackerman and Montalvo, 1990). The production of fruits per inflorescence is still the most used parameter to determine reproductive success in orchids (Ackerman, 1989b; Zimmerman and Aide, 1989; Ackerman and Montalvo, 1990). The *in vitro* germination of uniform samples, however, seems to be a useful way to determine the viability of seeds, since the presence of the embryo does not imply successful germination (P. C. Martini, unpublished data). Even though a few fruits were produced in the control group, seedlings of *G. quinquenervis* were not found in the study site or near it.

Phenology

The simultaneous opening of all flowers in an inflorescence is not common in orchids. An anthesis of 2 to 4 days is rare, since other species can last for many weeks. Those characteristics were observed on *G. quinquenervis*. Dodson and Frymire (1961) mention the same behaviour for *G. maculata* and *Stanhopea*, where flowers lasted only 2 days. The short longevity is probably influenced by the high energetic costs involved in perfume secretion (Gerlach and Schill, 1991). A synchronism

occurred in the inflorescence, but the overlap of flower-bearing individuals was very low, with a predominance of isolated flowerings. This strategy represents a high risk to small populations which might face decline since xenogamy is not favoured.

The high percentages of pollinaria removed by the male bees characterize the great male success of flowers. But the low percentage of pollinaria deposition on stigmas and, consequently, the low reproductive success was surprising, even though flower visitors were very abundant. Neiland and Wilcock (1998) suggested that the low reproductive success of tropical orchids could possibly be explained by the following factors: differences in population structure, which are usually small and widely dispersed (Ackerman, 1986) and competition for few pollinators. These authors even suggest that even in an orchid that offers perfume as a reward, like *Coryanthes* (Stanhopeinae), reproductive success would still be quite low when compared to a nectarless species from the temperate zones.

In this study, the very low reproductive success of *G. quinquenervis* could be mainly related to the size of the population. Besides, the study site is an area that has undergone great human influence until 40 years ago, and this human action continues in the vicinity, since the forest of the area is still being cut down to serve either as charcoal or for sugar-cane plantations.

We interpret the low reproductive success of *Gongora quinquenervis* as a consequence of a population in decline. Even with the abundant presence of pollinators, the persistence of this orchid in such degraded Atlantic Forest areas is questionable because of the small number of orchid individuals, in combination with the risk from its phenological characteristics which do not synchronize flowering phases within the population.

Acknowledgements

We thank Dr. Leonardo Pessoa Felix, Dr. Isabel Cristina Sobreira Machado and two anonymous referees for their valuable comments and suggestions on this manuscript, Roberto Siqueira Carneiro from the Refúgio Ecológico Charles Darwin for the support during the fieldwork. We also wish to thank CNPq for the financial support.

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P. Martini
Rua Jerônimo de Albuquerque, nº 86, Apt. 301-B
Casa Forte
Recife, PE 52061-470
Brazil
E-mail: pcmartini@hotmail.com

Section Editor: G. Gottsberger