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Auto-pollination in a long-spurred endemic orchid (*Jumellea stenophylla*) on Reunion Island (Mascarene Archipelago, Indian Ocean)

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Summary. Since Darwin, long-spurred angraecoid orchids have been known for their fascinating evolutionary relationship with long-tongued hawkmoths (Sphingidae) on Madagascar. We studied the reproductive biology of the long-spurred endemic *Jumellea stenophylla* on Reunion. Despite the species exhibits flowers with the typical sphingophilous pollination syndrome (i.e. spur length averaged 137.9 mm, mean nectar volume was 6.1 μl, and nectar concentration was 10.7% sugar in sucrose equivalent), it does not require pollinators to achieve fruits. Compared with other hawkmoth-pollinated orchids, flower longevity was very short, lasting less than 5 days, and the species did not emit the characteristic strong and sweet scent at dusk. Fruit set ranged from 66.7 to 83.9% when pollinators were excluded, and 56–77.5% under natural conditions. Auto-pollination is a consequence of structural modifications. On Reunion, such breeding system is not rare within long-spurred species, and seems linked to the absence of specific pollinator during island colonization, and species establishment.

Keywords: *Jumellea stenophylla*; long-spurred angraecoid orchid; oceanic island; Reunion Island; auto-pollination

Introduction

Auto-pollination (ability to reproduce without pollen vector, sensu Catling 1990) is widespread within the Orchidaceae (5–20% of species, Catling 1990), and its presence in many independent lineages indicates convergent evolution (Catling 1990; Dressler 1993). It is, however, more common in terrestrial species than in epiphytic orchids (Dressler 1993). Auto-pollination has been often explained as an evolutionary consequence of lack of pollinators or specialized pollinators, providing reproductive assurance when pollinator fauna is low, such as in boreal regions (e.g. Catling 1990), in cold mountain habitats (e.g. Dressler 1993) or in insular

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ecosystems (e.g. Barrett 1985, 1996; Jacquemyn et al. 2005).

Oceanic islands are known for the paucity of their insect fauna, and the absence of whole groups of insects (e.g. Carlquist 1974; Woodell 1979; Lloyd 1985; McMullen 1987; Barrett 1996). As a consequence, rates of auto-pollinated species (e.g. McMullen 1987; Webb and Kelly 1993; Barrett 1996; see also Schueller 2004 for more references; Jacquemyn et al. 2005), wind-pollinated species (e.g. Carlquist 1974; Barrett 1996; Anderson et al. 2001; Bernardello et al. 2001), or bird-pollinated species (e.g. Feinsinger et al. 1982; Anderson et al. 2001; Anderson 2003; Bernardello et al. 2004; Dupont et al. 2004) are often higher on islands compared with mainland areas.

The islands of Reunion, Mauritius and Rodriguez form the Mascarene Archipelago in the southern Indian Ocean, roughly 800–1,000 km east of Madagascar. Whereas Mauritius emerged at nearly 8 million years ago, Reunion is much younger, having emerged at roughly 2.1 million years ago (McDougall and Chamalaun 1969). Reunion is characterized by high orchid richness, comprising approximately 130 native species (du Petit-Thouars 1822; de Cordemoy 1895, 1899; Roberts 2001; see also Jacquemyn et al. 2005). Almost half of them are thought to be auto-pollinating (Jacquemyn et al. 2005), resulting in the island having one of the highest rates of auto-pollination recorded so far (Jacquemyn et al. 2005). On Reunion, angraecoid orchids (i.e. subtribes Angraecinae and Aerangidinae, Vandeae, Chase et al. 2003) represent the most species rich orchid lineage (ca. 52 spp.), with ten long-spurred species (i.e. spur length > 9 cm). While auto-pollination is thought to be rare within the Vandeae (sensu Dressler 1993) (0.3% of species, Dressler 1993), approximately 20% of angraecoid orchids on Reunion do not require an insect vector (Jacquemyn et al. 2005; Micheneau 2005).

Since the time of Darwin (1862), long-spurred angraecoid orchids have been known for their fascinating evolutionary relationship with long-tongued hawkmoths (Sphingidae) on Madagascar. The pollination systems of only a few species have, however, been studied so far. Nevertheless, all studies have shown that long-spurred angraecoid orchids were highly pollinator specific, as most have only a single recorded pollinator species or rarely two (Nilsson et al. 1985, 1987; Nilsson and Rabakonandrianina 1988; Wasserthal 1997; Martins and Johnson 2007). This specialization has been thought to be the result of a long-term evolutionary relationship between long-tongued sphingids and long-spurred orchids (Nilsson et al. 1985). Angraecoid orchids that are hawkmoth-pollinated exhibit typically long-spurred flowers (from 9 to 40 cm), pure white, producing relatively large amounts of nectar (reward), and have a sweet scent at dusk (attractant) (van der Pijl and Dodson 1966). Sphingids that pollinate such species have a long tongue, fly at dusk over long distances, locate the flower from long distances by the orchids scent, and at short distance by their white color. They visit the flower for the nectar it contains (van der Pijl and Dodson 1966; Nilsson 1992; see also Martins and Johnson 2007).

The purpose of this study was to describe the reproductive biology of the rare and endangered *Jumellea stenophylla* (Frapp.) Schltr., a long-spurred angraecoid orchid endemic to Reunion. The pollination biology of *Jumellea* has been investigated only once: in Madagascar, *J. teretifolia* Schltr. (with a spur length of about 12–13 cm) is pollinated by *Panogena lingens* Butl. (with a proboscis averaging 10.5 cm in length, Nilsson et al. 1987), a native hawkmoth of Madagascar, however, not present on Reunion. While previous observations indicated that *J. stenophylla* is able to auto-pollinate (Jacquemyn et al. 2005), we asked the following questions: (1) what are the floral features of *J. stenophylla* (spur length, floral scent, nectar properties)? Do these features fit the sphingophilous pollination syndrome? (2) What is the reproductive success in natural conditions? (3) By which mechanisms auto-pollination does occur? (4) Is *J. stenophylla* an obligated auto-pollinated species or does it use pollinator services to reproduce in natural populations?

Materials and methods

Study species and study site. The genus *Jumellea* Schltr. comprises about 60 species, which occur on Madagascar (42 species), the Comoros Islands (6 species), mainland Africa (2 species) (World checklist of Monocotyledons 2006), and the Mascarene Islands (9 species) (Fig. 1a). All species that are present on Reunion are endemic to the Mascarenes; five of them are long-spurred (spur length more than 9 cm) and four are short-spurred (spur length less than 5 cm). *Jumellea stenophylla* is an endangered, epiphytic species, endemic to Reunion. Each individual produces one or a small number of vegetative shoots that generally produce one or two solitary flowers per year. Flowers are white to brownish and long-spurred. *J. stenophylla* grows typically in *Pandanus* thickets, a habitat that has declined greatly since human colonization of Reunion (Strasberg et al. 2005). In this habitat, populations often contain fewer than 100 individuals, and less than 10 sites (all between 600–1,000 m asl) are known so far (Fig. 1b). The largest of the known populations in the wild was used for this study, located in the humid mid-altitude forest of Eden, in the eastern part of the island, at 650 m asl (Fig. 1b). In this habitat, individuals grow preferentially on *Pandanus montanus* Bory trees. At the study site, the flowering season lasted only two weeks, from mid November to early December.

Flower longevity, spur length, and nectar properties. To estimate flower longevity in natural condition, flowers were followed daily: newly opened flowers (chosen randomly in the population) were tagged with a different color each day. At the same time, a number of tagged flowers were collected for measurements following four different stages: (1) one-day-old flowers ($N = 10$) (hereafter D1), (2) two-day-old flowers ($N = 16$) (hereafter D2), (3) three-day-old flowers ($N = 6$) (hereafter D3), and (4) four-day-old flowers ($N = 12$) (hereafter D4) (Fig. 2). Spur length (distance between the tip of the spur and the entrance to the spur) was measured to the nearest 0.01 mm using digital calipers. Spur length measurements were performed on fresh material in the field. The same flowers were used to measure the height of the nectar column in the spur (distance between the tip of the spur and the level of nectar in the spur) with the same precision. To allow the collection of nectar, the tip of the spur was slightly opened using a scalpel. The nectar was then directly collected from the spur with a capillary tube (5 µl). The percent of sucrose equivalents in the nectar (gram of sucrose per 100 g of solution) was quantified by directly transferring the nectar from the capillary tube to a hand refractometer (R5000, Atago Inc., Bellevue, WA, USA).

Scent analysis. Floral volatiles were analyzed in 2004 and 2006, using solid phase micro extraction (SPME) technique (Zhang and Pawliszyn 1993). Two different SPME fibers were used: a black Carboxen/Polydimethylsiloxane (CAR/PDMS) fiber (length 1 cm, film thickness 75 µm) recommended for volatile compounds of low molecular weight and a gray StableFlex Divinylbenzene/Carboxen/

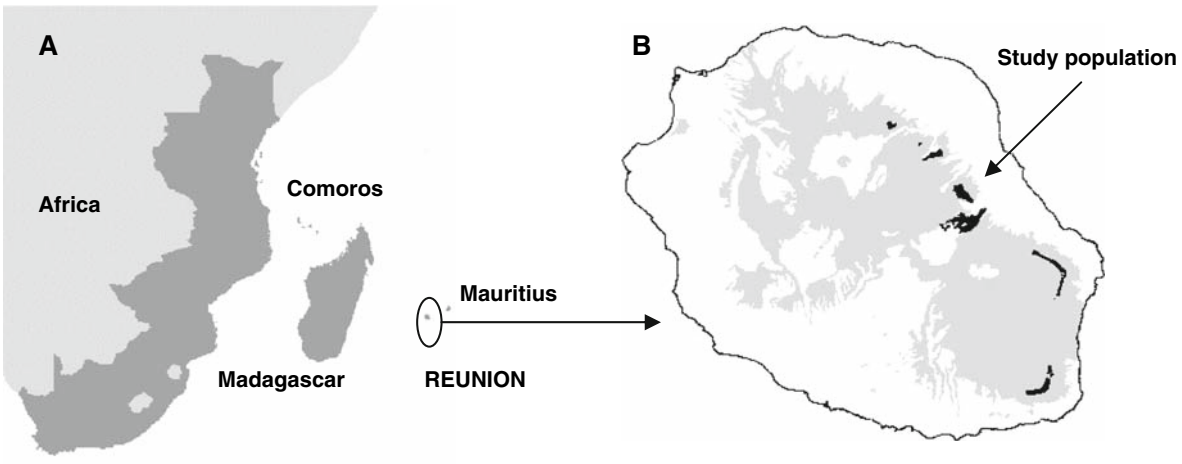

Fig. 1. Geographical distribution. **A** Worldwide distribution of the genus *Jumellea* (*dark zones*). **B** Repartition of *J. stenopylla* populations (*dark zones*) among remaining preserved habitats (*light zones*) on Reunion

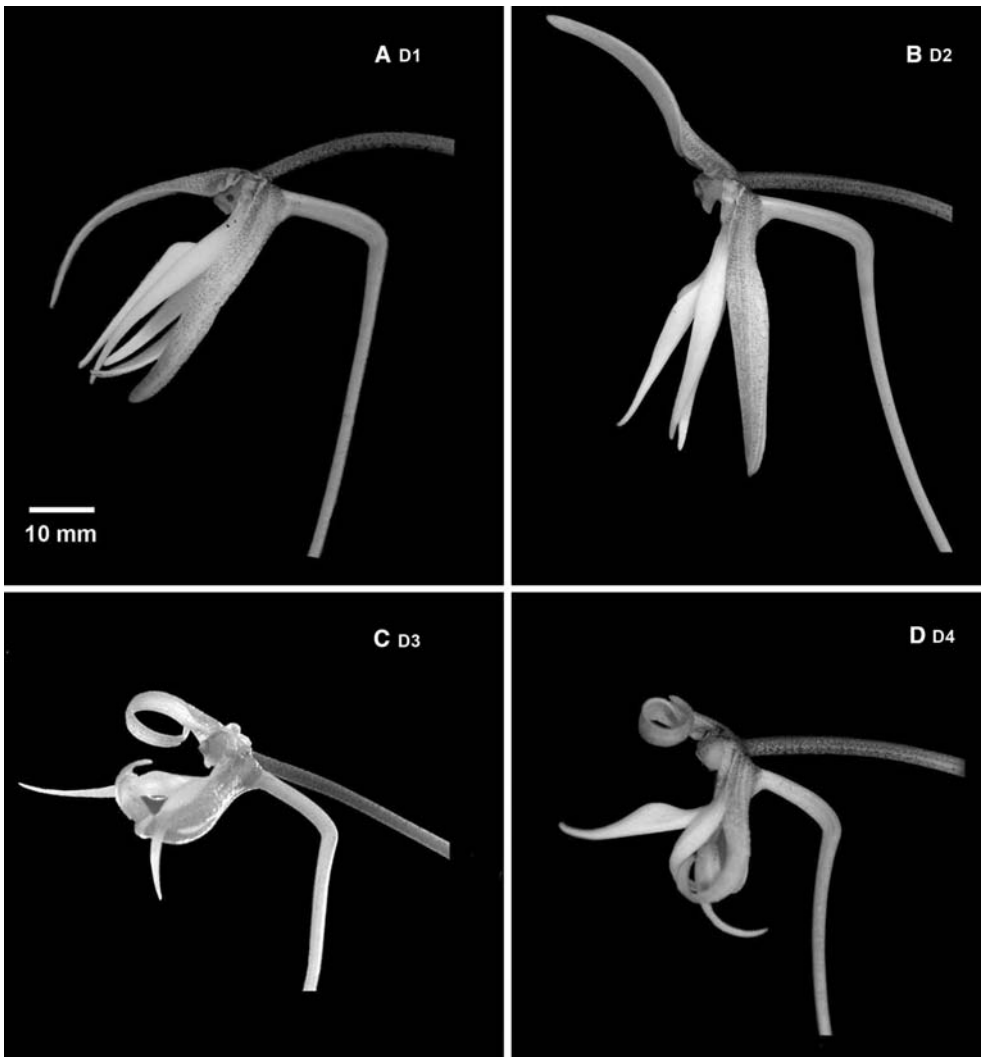

Fig. 2. Illustration of the different flower stages. **A** One-day old flowers (*D1*). **B** Two-day old flowers (*D2*). **C** Three-day old flowers (*D3*). **D** Four-day old flowers (*D4*)

Polydimethylsiloxane (DVB/CAR/PDMS) fiber (length 2 cm, film thickness 50/30 μm) indicated for flavors (volatiles and semivolatiles); both obtained from Supelco Co. (Bellefonte, PA, USA). The fibers were conditioned prior use according to supplier's prescriptions, 1 h at 300°C for CAR/PDMS, and 1 h at 270°C for DVB/CAR/PDMS. Before the first daily analysis the fibers were conditioned for 10 min.

Flowers were obtained from wild specimens, cultivated at the laboratory during the flowering period, and put back in the field after experiments. Two plants were collected at La Plaine des Palmistes (hereafter PP) (the first one in 2004, the second one in 2006), and the third plant at l'Eden (hereafter ED) in 2006. Floral analyses were achieved on living, intact flowers and began when flowers were 2-days old (D2 stage). They were placed in a glass-bell, sealed at the large extremity with cotton wool and at the other end by the SPME-fiber. For the three collected samples, diurnal and nocturnal fragrance productions were compared by collecting odor from the same flower during 9–10 h of daylight versus night, on two consecutive days (D2 and D3) to ensure the result reproducibility. SPME-fiber exposition conditions are summarized in Table 1. Blanks (i.e. exposed fiber without flower) were run to establish a base line. Analyses were performed using a Hewlett Packard

Table 1. SPME-fiber exposition conditions

Years	Pop	SPME-fiber coating	SPME-fiber exposition time			
			D2 flower stage		D3 flower stage	
			Day	Night	Day	Night
2004	PP	CAR/PDMS	8.00 am–5.30 pm	8.00 am–5.30 pm	5.30 pm–9.00 am	5.30 pm–9.00 am
2006	PP	DVB/CARB/PDMS	8.30 am–5.30 pm	8.30 am–5.30 pm	5.30 pm–8.30 am	5.30 pm–8.00 am
2006	ED	DVB/CARB/PDMS	8.30 am–5.30 pm	8.30 am–5.30 pm	6.00 pm–8.00 am	6.00 pm–8.00 am

Pop populations, *PP* Plaine des Palmistes, *ED* Eden

6890N gas chromatograph (GC), coupled directly to a Hewlett Packard 5973N mass spectrometer (MS). Compounds were desorbed from the fiber in the GC injector (splitless injection mode) at 250°C and separated on a capillary SPB-5 nonpolar column (60 m × 32 mm; phase thickness 0.25 μm) with helium as carrier gas (0.7 ml/min). The GC oven was programmed to increase temperature from 60 to 230°C at 4°C/min, following by a stabilization at 230°C during 40 min. Mass spectra were produced with a current ionization of 70 eV, in a scan range of *m*/z 30–550. Retention indices of the constituents were determined by Kovats method using *n*-alkanes (C_8–C_{22}) as standards (Kovats 1965). Compounds were identified by comparison of their retention indices and their mass spectral fragmentation with those reported in the literature (Adams 2001) and stored on the MS "Nist 2002" and "Wiley 7" libraries.

Breeding system and natural fruit set. The ability of the plant to reproduce by autonomous self-pollinations was tested in the field during 2003 ($N = 21$) and 2006 ($N = 31$) flowering seasons. Flowers, randomly chosen in the population, were enclosed in bags just prior to anthesis to exclude pollinators. Bags were maintained up to the end of the fruiting period to prevent predation. Natural fruit set was quantified in the same population in 2003 ($N = 40$ flowers), in 2004 ($N = 23$ flowers), and in 2006 ($N = 33$ flowers): unmanipulated flowers were marked just before anthesis. Fruit set was recorded for each treatment around 4 weeks after the flowering period; it was calculated by the ratio of mature fruits to open flowers. In 2004, 54 additional flowers were tagged, and examined for pollinaria removal in the studied population. Comparisons between bagged and opened pollinations were performed using Fisher's exact tests with no prior alternative, and opened pollination comparisons per year were performed using Kruskal–Wallis test. All statistical tests were performed using R software (version 1.10) for MacOSX (Iacus and Urbanek 2005).

Mechanism of auto-pollination. To describe auto-pollination mechanism of the species, longitudinal sections of the gynostem of the flowers collected at the different stages described above were observed under a Leica MZII stereomicroscope with a 6× magnification.

Results

Flower longevity, spur length, and nectar properties. The flower longevity was very short, as the flowers typically started to wilt at the end of the fourth day (D4 stage). Spur length increased over blossoming time to reach its maximum size on 4-day-old flowers (D4 stage) (Table 2). Contrarily to the spur length, nectar volume and sugar concentration continuously decreased until the flowers wilted (Table 2).

Scent analysis. The results obtained depend both on the experimental conditions (day or night) and on the properties of the fiber coating.

First, concerning the experimental conditions, whatever the fiber coating, day and night analyses showed approximately identical results, with the nitrogenous compound indole as unique dominant compound by far. However, nocturnal emissions were clearly higher. Nocturnally, emitted fragrances gave well-resoluted GC chromatograms (Fig. 3) with peaks of high intensity whereas those emitted during the day gave GC chromatograms much less clear marked by the lack of some minor compounds. All these experiments allow us to conclude that

Table 2. Mean ± SD (*N*) of spur length and nectar properties over flower lifespan

	Flower stages			
	D1	D2	D3	D4
Spur length (mm)	114.6 ± 21.8 (10)	128.5 ± 17.6 (16)	129.9 ± 4.1 (6)	137.9 ± 9.0 (12)
Nectar				
Height (mm)	41.7 ± 42.2 (10)	26.2 ± 18.5 (16)	27.6 ± 15.3 (5)	3.2 ± 9.7 (12)
Volume (μl)	6.1 ± 6.1 (9)	2.9 ± 3.2 (16)	1.6 ± 0.7 (4)	0.2 ± 0.8 (12)
Sugar (g/100 g)	10.7 ± 1.3 (6)	9.2 ± 1.4 (2)	7.6 ± 1.8 (6)	5.2 (1)

J. stenophylla emits fragrance on a nocturnal rhythm. For this reason, only volatile compounds detected during the night are reported in Table 3.

Second, concerning the fiber coating types, it also clearly appeared that the use of DVB/CAR/PDMS led to more effective trapping of volatile compounds; the CAR/PDMS fiber being more selective. Indeed, when comparing the results for the three samples, 14–16 minor compounds (depending on plant location) were extracted by the DVB/CAR/PDMS fiber whereas only five minor components were detected by the use of the CAR/PDMS fiber.

In conclusion, the present results demonstrated that the best conditions for volatile compounds extraction of *J. stenophylla* were performed at night with the DVB/CAR/PDMS fiber. In these conditions, *J. stenophylla* fragrance is largely dominated by indole (83.3–86.6%), accompanied by lower contents of hydrocarbons, aldehydes, ketones, mono- and sesquiterpene hydrocarbons and aromatic compounds including benzaldehyde (3.4–3.9%), methylbenzoate (0.7–0.9%) and benzylbenzoate (0.4–0.9%).

Breeding system and natural fruit set. *Jumellea stenophylla* is clearly able to reproduce in the absence of pollinators. The fruiting success of bagged flowers reached 66.7% in 2003 ($N = 21$) and 83.9% in 2006 ($N = 31$), while fruit production under natural conditions was 77.5% ($N = 40$) in 2003, and 54.5% ($N = 33$) in 2006. In 2003, fruit set did not significantly differ whether pollinators were excluded or not ($P = 0.3764$), but it was significantly different in 2006 ($P = 0.0155$), with fruit set of bagged flowers superior to fruit set in natural conditions, suggesting that pollinators play a minor, if any, role in fruit production under natural conditions. In 2004, the natural fruit set (56.5%, $N = 23$) was lower than in 2003, but slightly higher than in 2006; however, no significant difference between years has been detected ($P = 0.0648$). None of the 54 additional observed flowers had removed pollinarium in 2004.

Mechanism of auto-pollination. Column observations at D1 flower stages revealed that there is no rostellum that separated pollinia from stigmatic surface (Fig. 4a). Pollinia, which are attached laterally to each column side, are protected by the anther cap, which is fused to the column.

At D2 stages, pollinia increased in size, indicating a vacuolated phase (Fig. 4b). Typically, vacuolization occurs after pollination and before pollen tube growth (Pacini and Hesse 2002). The anther cap remained fused to the column.

Column longitudinal sections of D3 and D4 flower stages showed approximately identical patterns: column tissues developed, the upper part bent, and enclosed totally pollinia, which filled the stigmatic cavity quasi-totally, and started to disaggregate (Fig. 4c, D3 stage not shown). Again, the anther cap remained attached to the column.

Discussion

Flower longevity, spur length and nectar properties. In our study, different floral traits related to plant-pollinator interaction were investigated, namely, flower longevity, spur length, nectar properties, and scent production.

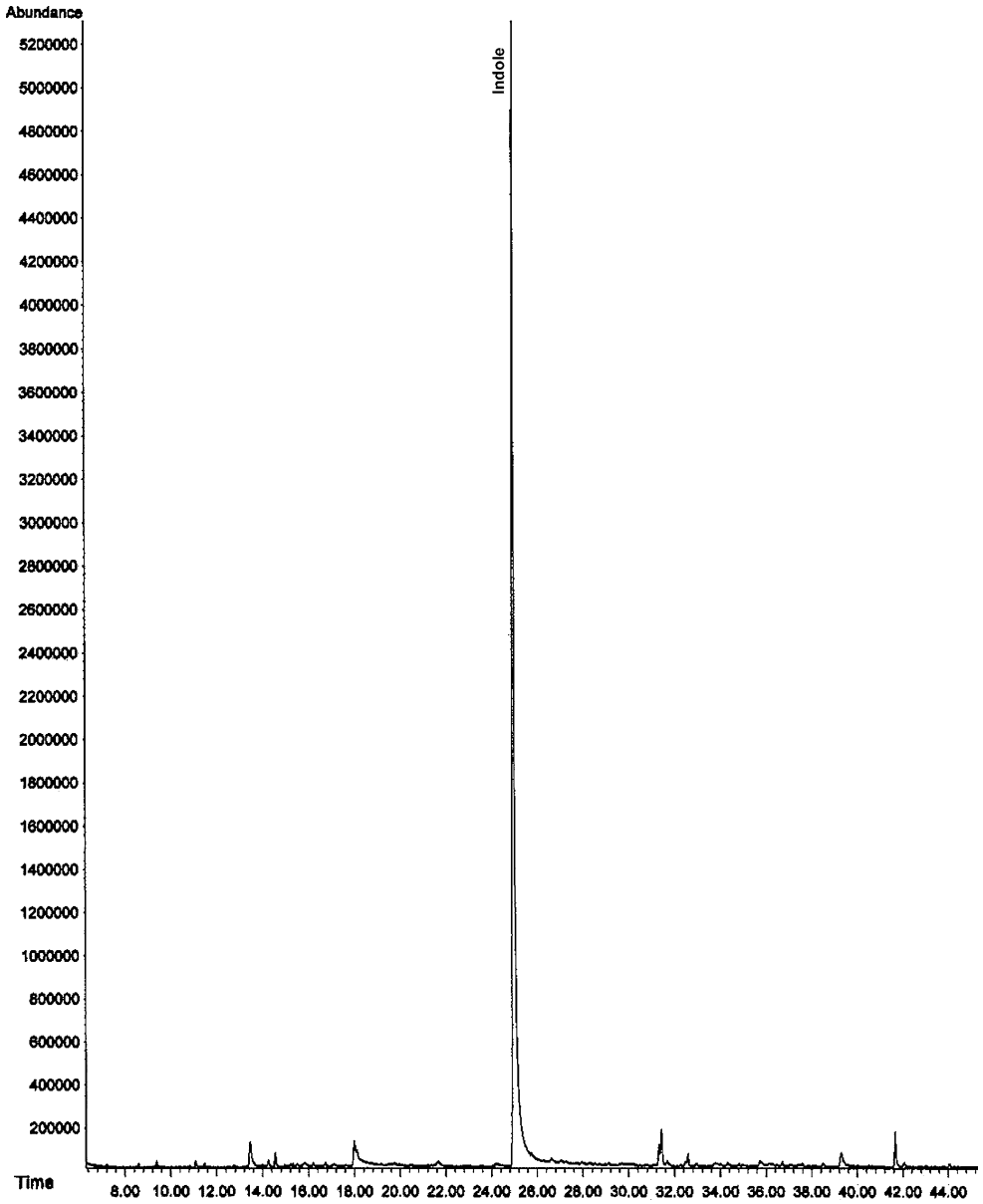

Fig. 3. Night GC chromatogram of *J. stenophylla* (D3 flower stage, plant from Plaine des Palmistes, December 2006)

Flower longevity of *J. stenophylla* was found to be very short (only 4–5 days), compared with those of other hawkmoth-pollinated orchids. For example, the lifespan of flowers of *Mystacidium venosum* Harve ex. Rolf (Aerangidinae, Vandeae) last 24 days on average, but blooming was reduced to 19 days when pollinaria were removed and decreased to 5 days when the flower was pollinated (Luyt and Johnson 2001). The shortened flower longevity of *J. stenophylla* seems correlated with autonomous self-pollinations that occurred in all flowers, even if a fruit may not necessarily develop. Spur length of *J. stenophylla* (137.9 mm) ranged within the reported spur size of long-spurred angraecoid orchids studied so far (e.g. Nilsson et al. 1987; Wasserthal 1997; Martins and Johnson 2007), but it produced a relatively small amount of nectar

Table 3. Night headspace SPME volatiles of D3 stage *J. stenophylla* flowers

Compounds	RRI[a]	Percentage[b]		
		PP 2004	PP 2006	ED 2006
Hydrocarbons				
Nonane	912	–	0.3	–
Decane	1011	–	1.9	tr
Heptadecane	1712	–	1.0	tr
Aldehydes				
Dodecanal	1422	–	–	1.8
Ketones				
6-Methyl-5-Hepten-2-one	997	–	–	0.3
Geranyl actetone	1467	–	–	0.5
Aromatic compounds				
Benzaldehyde	977	8.5	3.4	3.9
Benzyl alcohol	1047	1.5	tr	0.7
p-Cymenene	1106	–	–	0.3
Methyl benzoate	1111	1.1	0.9	0.7
trans-Calamenene	1552	–	tr	–
Benzyl benzoate	1797	–	0.4	0.9
Monoterpenes				
Isocitronellene	947	–	0.5	–
E-β-Ocimene	1059	–	tr	0.2
cis-Linalool oxide (pyranoid)	1177	0.3	–	–
Sesquiterpens hydrocarbons				
α-Copaene	1404	–	1.1	0.7
α-Isocomene	1415	–	0.7	–
trans-Muurola-4(14),5-diene[c]	1513	–	–	2.4
Z-β-Bizabolene	1525	–	tr	tr
δ-Cadinene	1551	–	–	tr
Nitrogenous compounds				
Indole	1314	88.4	85.6	83.3
Unidentified				
Compound 1	1023	–	0.5	0.4
Compound 2	1208	0.2	–	–
Compound 2	1353	–	2.1	1.3
Compound 3	1374	–	1.1	0.8
Compound 4	1379	–	0.5	0.4
Compound 5	1595	–	–	0.5
Compound 6	1685	–	–	0.9

[a] Relative retention indices to C_8–C_{22} *n*-alkanes on the SPB-5 column

[b] Relative percentage based on the peak area from the GC analysis; tr, <0.1%; –, absent

[c] Tentatively identified

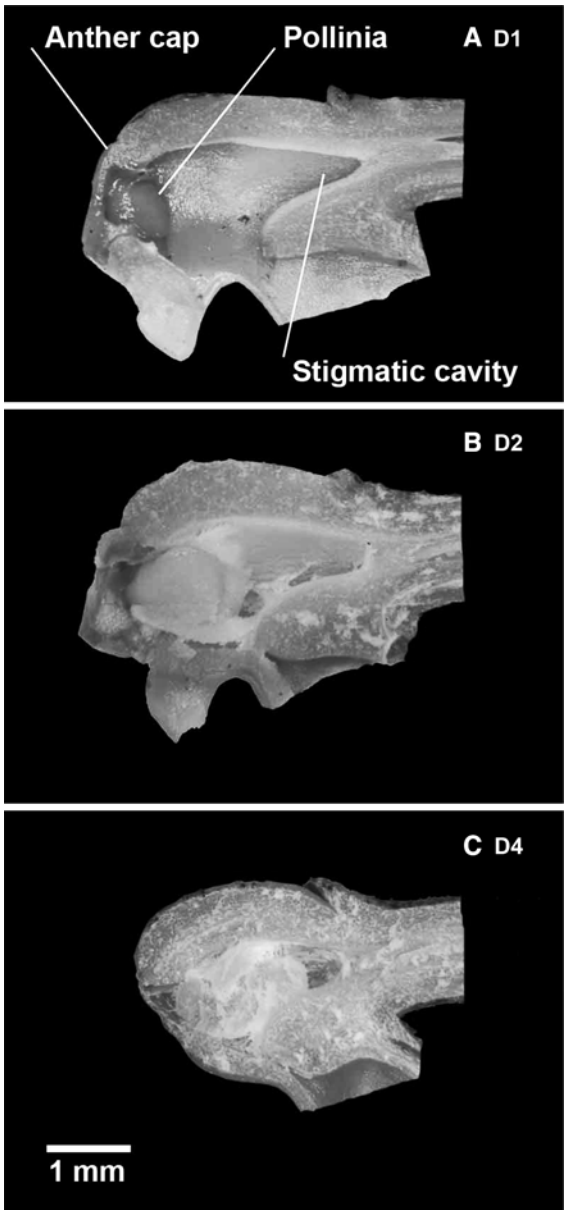

Fig. 4. Longitudinal sections of *J. stenophylla* column at different flower stages. **A** One-day old flowers (*D1*). Note the absence of rostellum between pollinia and stigmatic cavity. **B** Two-day old flowers (*D2*). Note the increased size of pollinia (vacuolization). **C** Four-day old flowers (*D4*). Note the development of column tissues

(6.1 µl). For example, Nilsson et al. (1985) found a nectar production of 12.8 µl in *Angraecum arachnites* Schltr. (Madagascar), and Martins and Johnson (2007) a nectar volume of 14.9 µl in

Aerangis thomsonii (Africa), two long-spurred species, which exhibit spurs quasi similar in length than those of *J. stenophylla*. Contrarily to nectar volume, nectar sugar concentration of *J. stenophylla* (10.7%) fell within the range reported in other hawkmoth-pollinated angraecoid orchids, which varied from 5 to 24% sugar in sucrose equivalents, with a mean value of 14.7% (SD = 5.7; $N = 7$) (data from Nilsson et al. 1985, 1987; Nilsson and Rabakonandrianina 1988; Wasserthal 1997; Luyt and Johnson 2001).

We observed that both nectar volume, and sugar concentration decreased over flower life-span. In the absence of pollinator, this pattern could be a consequence of nectar resorption by the flower. Nectar resorption has been reported in some angraecoid orchids (Koopowitz and Marchant 1998; Luyt and Johnson 2002). Luyt and Johnson (2002), for example, have shown that the resorption of nectar after pollination (and presumably its carbohydrate content) enhanced fruit size and seed viability of *M. venosum*. In *Platanthera chlorantha* (Custer) Rchb., sucrose originating from nectar resorption of pollinated flowers was found back in the initiated fruit (Stpiczyńska 2003). In the case of *J. stenophylla*, further studies are nevertheless needed; nectar resorption remains a hypothesis that needs to be proven.

Scent analysis. Orchid flower scent is known to have an important role in attracting pollinators at long distance. Hawkmoth-pollinated orchids give off a typical "white floral" night-scents (Kaiser 1993), also emitted by jasmine, honeysuckle, tuberose, lilies, or *Gardenia*, and night-scented *Nicotiana* species as well (Kaiser 1993). This "white floral" bouquet incorporates usually many different volatiles (up to 100) from different chemical classes, belonging mainly to acyclic terpene alcohols (primarily linalool, nerolidol, farnesol, including the corresponding hydrocarbons), aromatic alcohols (particularly benzyl alcohol, phenylethyl alcohol, and derived esters), and nitrogenous compounds (such as indole and oximes) (Kaiser 1993; Raguso et al. 2003). In *J. stenophylla*, our results have shown that its floral bouquet is primarily dominated by indole, nitrogenous compound which may be responsible of the extremely diffusive and powerful nocturnally fragrance of the flower (Arctander 1994).

This volatile compound is present in the majority of "white floral" night-scents, but its proportion is variable according to the age of the flower: (1) in young flowers (i.e. until 3 or 4 days old), it is often the dominant compound (Kaiser 1993); (2) in fully-developed flowers, it is however, usually found in very low proportions, and in mixture with other molecules (Kaiser 1993). The floral bouquet of *J. stenophylla* corresponds typically to young night-scent flower olfactory profile, which is consistent with the fact that our analyses were made on young flowers (i.e. D2 and D3 stage), just before auto-pollination induced flower senescence.

Breeding system and natural fruit set. Auto-pollination in *J. stenophylla* may be the result of two different scenarios: either ancestral newcomers of *J. stenophylla* were already able to auto-pollinate prior to Reunion colonization, or reproduction by autonomous self-pollination evolved in situ on Reunion. In the first case, auto-pollination may have facilitated establishment of pioneer individuals on Reunion (Baker 1955, 1967; Stebbins 1970). Due to difficulties of island long-distance colonization (Carlquist 1974), erratic newcomers may experience difficulties in finding both appropriate reproductive partners (especially in the extreme case of colonization by a single individual), and/or appropriate pollen vectors (especially in case of entomophilous pollination, since oceanic islands are known for their underrepresented insect fauna) (e.g. Carlquist 1974; Woodell 1979; McMullen 1987; Barrett 1996; Anderson et al. 2001). Selection for auto-pollination is then not rare for successful establishment of newcomers to isolated islands (Baker 1955, 1967; Stebbins 1970; see also Barrett 1996; Schueller 2004). While "strength, mechanism, and timing of selection for selfing is likely to depend on the initial breeding system of colonists and on the nature of island pollinators" (Schueller 2004), we could hypothesize that absence of specialized pollinators on Reunion during species establishment, compared to the

Malagasy situation, may have led to autochthonous evolution to auto-pollination. However, evolution processes for ability to auto-pollinate are not well known, especially the inference of lack of mates on individual selection for auto-pollination (Schueller 2004). According to Catling (1990) "auto-pollination in orchids has a genetic basis with little environmental control".

Fruit set of auto-pollinated orchid is generally high, averaging 77.0% (Tremblay et al. 2005). For *J. stenophylla*, natural fruit set of was close to this value, ranging from 53.6 to 77.5%. The pollination biology of *Jumellea* genus has been investigated only once (i.e. *J. teretifolia*, Nilsson et al. 1987), but fruit set in natural conditions was not reported.

Mechanism of auto-pollination. Several auto-pollination mechanisms have been described in the Orchidaceae (see Catling 1990 for review; see also Ke-Wei et al. 2006), including morphological structural modifications, or bending of caudicules, which bring the pollinia onto the stigma (sometimes rain-assisted, Catling 1980, 1990). For *J. stenophylla*, auto-pollination is under structural column modifications: the absence of a true rostellum allows auto-pollination very early during the development stage of the flower. Moreover, pollinarium removal seems impossible at any time of the flower lifespan. Functionally, structural column modifications imply that *J. stenophylla* is an obligated auto-pollinated species.

Although auto-pollination has mostly been described as a consequence of a bending caudicule in the Vandeae (sensu Dressler 1981; Catling 1990), we report in this study a case of auto-pollination via structural modifications for this tribe. Auto-pollination as a consequence of structural modifications has been suggested by Bosser (1988) for another Reunion endemic angraecoid orchid, *A. borbonicum* Bosser (spur length of ca. 6.5–6.9 cm, Bosser 1988; Micheneau 2005), but further studies are needed to describe more precisely the auto-pollination process.

Concluding remarks. Auto-pollinated orchids are often characterized by reduction or loss of floral traits associated with plant–pollinator interactions. These convergent and predictable features include small flower size, small contrasting color patterns, absence of nectar guide, absence of odor, absent or reduced spur (see Catling 1990). Similar patterns (loss of floral adaptations related to pollination) have been reported for other auto-pollinating insular plants species (see Barrett 1996). In the case of *J. stenophylla* consequences of auto-pollination on floral morphology include adaptations that favor auto-pollination success (column modifications), but both spur length and nectar production are closed to those recorded for long-spurred hawkmoth-pollinated angraecoid orchids from Madagascar. While flowers do not emit strong and sweet scent at dusk as it was reported for hawkmoth-pollinated angraecoid orchids (e.g. Martins and Johnson 2007), this seems likely to be due to a consequence of flower lifespan brevity.

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