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Orchid–bird interactions: a case study from *Angraecum* (Vandaeae, Angraecinae) and *Zosterops* (white-eyes, Zosteropidae) on Reunion Island

Claire Micheneau, Jacques Fournel, Laurence Humeau, and Thierry Pailler

Abstract: Although angraecoid orchids are famous for their spectacular flowers, pollinated by hawkmoths in Madagascar, two species of *Angraecum* endemic to the Mascarenes (sect. *Hadrangis*) have atypical unscented and short-spurred flowers that are pollinated by birds. We focused on the pollination and reproductive success of *Angraecum bracteosum* Balf. f. & S. Moore, a member of sect. *Hadrangis*. We investigated the breeding system by performing a set of hand-pollination experiments and by observing natural pollinators using video-captures. Pollinator efficiency (pollen removal and deposition) and reproductive success (fruit set) were quantified once a week in two natural island populations during three consecutive flowering seasons (2003–2005). The orchid is self-compatible but requires pollinator services to achieve reproduction. The two white-eye song-bird species (Zosteropidae) that occur on Reunion, *Zosterops borbonicus* Boddaert and *Zosterops olivaceus* L., were recorded as pollinators. However, while pollination events were rare and occasional following flower visits by *Z. borbonicus*, foraging of *Z. olivaceus* resulted in a high frequency of successful pollinations. White-eyes typically perched on inflorescences, and probed most of the “fresh-looking” flowers for nectar. This study documents a probable case of bird-pollination specialization within *Angraecum* section *Hadrangis*, and sheds light on the role played by pollinators during the radiation of section *Hadrangis* in the Mascarene Archipelago.

Key words: angraecoid orchids, *Angraecum bracteosum*, bird-pollination, Mascarene Islands, white-eyes, Zosteropidae.

Résumé : Bien que les orchidées angraecoïdes de la région malgache soient réputées pour leur pollinisation par les lépidoptères nocturnes, deux espèces endémiques des Mascareignes, appartenant à la section *Hadrangis* du genre *Angraecum* présentent des fleurs bien particulières, pollinisées par des oiseaux, avec un court éperon et dépourvues d'odeur. Nous avons axé notre étude sur la pollinisation et le succès reproducteur d'*Angraecum bracteosum* Balf. f. & S. Moore, l'une des trois espèces de la section *Hadrangis*. Le système de compatibilité a été étudié par une série de pollinisations manuelles et l'observation des pollinisateurs a été faite au moyen d'une caméra vidéo. Une fois par semaine et au cours de trois années consécutives (de 2003 à 2005), l'activité des pollinisateurs (prélèvements et dépôts de pollen), ainsi que le succès reproducteur (production de fruits) ont été quantifiés dans deux sites d'étude. *A. bracteosum* est auto-compatible, mais l'espèce dépend de pollinisateurs pour se reproduire. Les deux espèces d'oiseaux « lunettes » présentes à la Réunion, *Zosterops borbonicus* Boddaert et *Zosterops olivaceus* L. (Zosteropidae) pollinisent l'orchidée. Cependant, alors que les événements de pollinisation consécutifs aux visites de *Z. borbonicus* sont rares et occasionnels, la pollinisation est bien plus efficace lors des visites de *Z. olivaceus*. Les deux espèces d'oiseaux adoptent le même comportement lors de la visite des fleurs : ils se perchent sur les inflorescences de l'orchidée et se nourrissent du nectar de la majorité des fleurs à leur disposition. Cette étude documente un cas probable de spécialisation dans la pollinisation par les oiseaux des espèces de la section *Hadrangis* du genre *Angraecum*. Ceci soulève le rôle des pollinisateurs dans la radiation de la section *Hadrangis* aux Mascareignes.

Mots-clés : orchidées angraecoïdes, *Angraecum bracteosum*, ornithophilie, îles des Mascareignes, Zosteropidae.

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Introduction

Within the paradigm of flower pollination syndromes, the large genus *Angraecum* (Orchidaceae) provides the best example of deep tube flower adaptation to giant moth pollination (i.e., *Angraecum sesquipedale* Thou., Darwin 1862; Nilsson 1988). The genus has, however, revealed surprises when it was recently discovered that one species, *Angraecum striatum* Thou., endemic to the island of Reunion, is pollinated by a small song-bird, the grey white-eye, *Zosterops borbonicus* Boddaert subsp. *borbonicus* (Zosteropidae) also endemic to Reunion (Micheneau et al. 2006).

Angraecum striatum (Reunion) along with *Angraecum bracteosum* Balf. f. & S.Moore (Reunion) and *Angraecum cadetii* Bosser (Reunion and Mauritius) constitute the Mascarene endemic section *Hadrangis*; *A. striatum* and *A. bracteosum* display a bird pollination floral syndrome (i.e., unscented short-spurred flowers containing a large amount of dilute nectar). As a clade entirely endemic to the Mascarenes, the section represents one of the rare cases of an *Angraecum* intra-archipelago radiation (Micheneau et al. 2008), raising the intriguing and exciting question of how they speciated. On Reunion, sect. *Hadrangis* is well represented (except *A. cadetii* which is rare), and the three species can grow in sympatry, sometimes on the same host tree (personal observation). However, section *Hadrangis* flower morphology is divergent among species, and hybrids do not seem to occur in the wild (personal observation). Since bird pollination by grey white-eyes (*Z. borbonicus* subsp. *borbonicus*) was observed for *A. striatum*, determining whether other birds (especially the nectar-specialized olive white-eye, *Zosterops olivaceus* L. subsp. *olivaceus*, the second white-eye species occurring on Reunion) were involved in the pollination of sect. *Hadrangis* appeared an essential preliminary step in understanding the sect. *Hadrangis* radiation on Reunion.

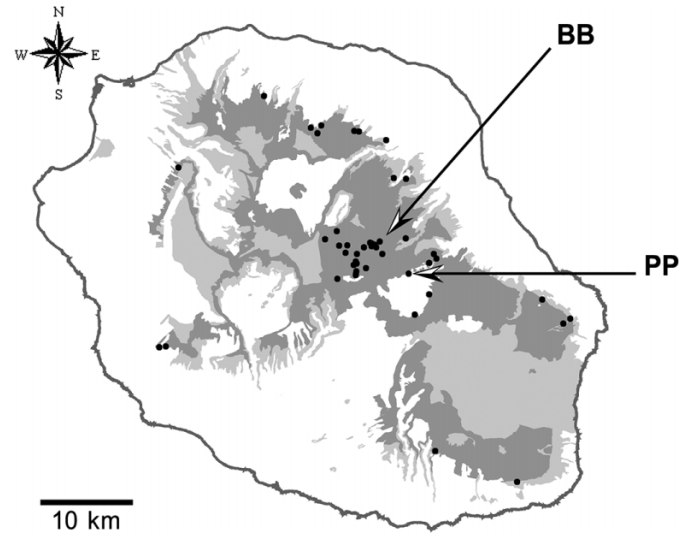
In this study, we focused on pollination and reproductive success of *A. bracteosum*, a member of sect. *Hadrangis*. The main objectives were (i) describe the breeding system of *A. bracteosum*, (ii) identify its natural pollinators, and (iii) quantify pollinator efficiency (pollen removal and deposition) and reproductive success (fruit set) in natural conditions. In identifying pollinators of *A. bracteosum*, we seek to improve our understanding of the factors driving the radiation of section *Hadrangis* on Reunion.

Materials and methods

Study species

Angraecum bracteosum is a monopodial epiphytic orchid with a short stem and distichous leaves. It is endemic to the island of Reunion, and is relatively common in primary forests from the lowlands to mountain cloud forests. Plants usually produce 1–7 compact racemes of 2–10 pure-white unscented flowers, fleshy in texture, with a prominent bract (13 mm in average length, being longer than the ovary). Flowers are medium size for the genus, with floral parts (except the lip) quite similar in both shape and size (ca. 13–15 mm in length, and 5–7 mm in width). The lip (ca. 9 mm wide and 13 mm long) adjoins a saccate and short spur (averaging 8 mm in length with an external diameter at the entrance of approximately 4 mm), containing a small amount of nectar. *Angraecum bracteosum* is the only species of sect. *Hadrangis* to display a strong fold on the lip (callus), restricting the flower's opening, which is consequently very reduced (distance between the lip and the pollinarium averages 1.9 mm). The pollinarium consists of two hard, pale-yellow, small pollinia, attached to a single-piece viscidium, so that both pollinia are removed simultaneously by potential pollinators. Pollinaria removal and deposition are easily observable in the field, providing the observer looks carefully under the anther cap, which can remain at-

Fig. 1. Distribution of *Angraecum bracteosum* on Reunion (dark zones) among remaining preserved habitats (light zones, from Strasberg et al. 2005) (dots represent precise localities where the species was encountered among 121 recorded sites, see Jacquemyn et al. 2005). BB, Bébou study site (cloud forest, 1150 m a.s.l.); PP, Plaine des Palmistes study site (*Pandanus* wet thickets, 800 m a.s.l.).



tached to the column even if pollinia have been removed by a pollinator.

Study site

Reunion (ca. 2500 km²) is an island located 800 km to the east of Madagascar (21°00'S, 55°39'E). It is a mountainous oceanic island, dominated by two volcanic massifs, Piton de la Fournaise (2619 m a.s.l.) (one of the most active volcanos in the world) and Piton des Neiges (3070) (the highest point in the Indian Ocean). Across this high elevational gradient, Reunion harbors a rich variety of habitats (from savanna to subalpine vegetation) (see Strasberg et al. 2005). Thanks to this complex topography, most habitats in the rugged interior of Reunion have been well preserved, contrary to lowland forests and littoral zones.

Our study was conducted at two different localities in the center of the island, the mountain cloud forest of Bébou (hereinafter BB) and the *Pandanus* thickets of the Plaine des Palmistes (hereinafter PP) (Fig. 1), chosen for the quality of the habitat preservation and the large sample size of *A. bracteosum* populations. The study site of BB is located at 1200 m. The main forest (among the most diverse and the best preserved of the island) consists of an assemblage of both endemic tree ferns and hardwood species, being refuge to an unusual richness of bryophytes and epiphytic orchids. At this site, *A. bracteosum* grows in sympatry with *A. striatum*, one of the three species of sect. *Hadrangis*. The study site of PP is a moist forest dominated by *Pandanus montanus* Bory. trees, situated in a mid-elevational zone at approximately 800 m in the vicinity of the Plaine des Palmistes. This unique habitat, endemic to Reunion, is home to an exceptional richness of orchids, including rare and endangered species such as *Angraecum conchoglossum* Schltr., *Oeonia rosea* Ridl., and *Jumellea stenophylla* Schltr.

At PP, the three sect. *Hadrangis* species are occurring in sympatry.

Nectar properties

Nectar properties were recorded on 19 unvisited flowers from 7 plants from the PP site in 2007. For each flower, we quantified nectar volume using calibrated capillary tubes of 5 μL . Nectar was then directly transferred to a hand refractometer (R5000; Atago USA, Inc., Bellevue, Wash.), from which the sugar concentration (g of sucrose equivalents $\cdot 100\text{ g}^{-1}$ of solution) was determined using sucrose equivalent tables, available in Mathlouthi and Reiser (1995).

Breeding system and compatibility

Hand-pollination experiments were set up both in situ (in 2005) and ex situ (in 2003) to investigate the breeding system of *A. bracteosum*, following the protocol described by Micheneau et al. (2006): in situ experiments were carried out on 10 individuals at the PP study site (17 inflorescences, 111 flowers). Prior to flower anthesis, plants were first enclosed by fine-meshsize nylon (i.e., strands per half millimetre) to exclude pollinaria removal and deposition by any potential pollinators. Three treatments were performed: (i) no pollination, to detect for repeatability this species' ability to set fruit in the absence of pollinators (auto-pollination sensu Catling 1990) (38 flowers, 3 plants), (ii) self-pollination to quantify self-compatibility (38 flowers, 2 plants), (iii) cross-pollinations (35 flowers, 5 plants). Self-pollinations were carried out by hand, pollinating flowers with their own two pollinia. Cross-pollinations were performed by hand, pollinating flowers with two pollinia from a distinct conspecific plant (more than 2 m away, as a minimum). Bags were maintained up to the end of the fruiting period to prevent predation.

The same sets of experiments were performed ex situ on 5 plants from PP (13 inflorescences, 62 flowers) cultivated in an open-air greenhouse (where plants were misted with water for 3 min every 6 h) as follows: 50 flowers (5 plants) were unmanipulated, 5 flowers (5 plants) were self-pollinated, and 7 flowers (5 plants) were cross-pollinated.

In situ, each inflorescence received the same treatment, and pollinations were performed on all the flowers of the inflorescence. This high level of pollen was also used to determine whether fruit set is limited by pollen availability (i.e., pollinator activity) versus resource allocation. Ex situ treatments, however, were assigned randomly on a few flowers per inflorescence to avoid resource bias. Fruit set was recorded for each treatment 4 weeks after pollination, when capsules reached their maximum size.

Pollinator observations

All pollinator observations were set up in situ using video-captures, following the protocol of Micheneau et al. (2006) for observations made in 2005. In 2007 and 2008, video data acquisition was improved: pollinator observations were performed using hard disk camcorders with night option (Sony DCR-SR90E, and Sony DCR-SR72E) fixed on a tripod, with power supplied by long-duration rechargeable batteries (NP-FP90, NP-NH100 InfoLithium[®] P and H Series Rechargeable Battery). Observations were recorded from

around 0615 to 1830, with a maximum of nonstop filming of 5.5 h (dictated by battery capability). Approximately every half day, the camera was removed for 1 h from the field, and temporarily replaced with a digital video camera (Sony DCR-TRV16E, equipped with 1 h Fuji DVM60 tapes) to transfer data recorded on the first camera to the hard drive of a laptop computer. To avoid weather dependence, the camcorder was protected with a waterproof casing (Sony SPK-HCB Marine Sport Pack). During each session, the camcorder, which was focused on one individual, was hidden in trees at least 5 m from the target individual. Before and after each videotape session, each flower of the target individual was examined for pollen removal and (or) deposition. Pollinators were identified from video-captures.

Pollination success and fruit set

During three consecutive flowering seasons (2003–2005), additional individuals were examined once a week (approximately) at both study sites (30 plants in BB and 20 plants in PP), for purposes of determining (i) male and female pollination success (pollen removal and deposition, respectively), and (ii) reproductive success (fruit development). Pollen removal rate was calculated with the following formula:

$$\% \text{ pollen removal} = [(1/N) \times \sum_{i=1}^N (n_r/n_t)_i] \times 100$$

with N = number of plants, n_r = number of flowers with removed pollinia, and n_t = total number of flowers per plant. Pollen deposition and fruit set were calculated following the same formula.

In 2003, each study plant was permanently tagged and mapped to the nearest metre in both sites to allow a perennial study through the years. We recorded for each tagged plant: host tree species, host tree diameter, and epiphyte height on the host tree to the nearest centimetre.

Data analyses

All statistical analyses were performed using R software (version 2.5.1) (R Development Core Team 2006). Pollination and reproductive variations between years and populations were analyzed using non-parametric Kruskal–Wallis χ^2 squared approximation and Wilcoxon's tests (Sokal and Rohlf 1995) applying Sidak's multiplicative inequality (Sokal and Rohlf 1995) for all pairwise combinations to account for multiple comparisons. To examine the effect of host species, host diameter, and epiphyte height on pollen deposition, pollen removal, and fruit set, Kruskal–Wallis χ^2 tests were applied. To test for any correlation between pollen deposition, pollen removal and fruit set, Pearson product moment correlations were conducted among all possible character combinations for each population and for each year. Significance levels for multiple comparisons were adjusted by the Bonferroni sequential technique following Rice (1989).

Results

Nectar properties

Nectar volume averaged 4.9 μL per flower (SD = 3.7; N = 19) with a concentration of 9.6% sugar in sucrose equivalents (SD = 1.3; N = 19).

Breeding system

None of the flowers tested for autonomous self-pollination produced fruit, either in situ or ex situ, suggesting that *A. bracteosum* is not able to set fruits without a pollen vector (Table 1). However, the species is fully self-compatible (Table 1), as the fruit set in cases of self-pollination reached 80% as a minimum (only one flower did not develop fruit either in situ or ex situ). In situ, recorded fruit set was extremely high (97% and 100% for self- and cross-pollination treatments, respectively), and all fruits were well developed (no abortions), whatever the position on the inflorescence, indicating that resources are not a factor limiting reproduction for this species at the PP site.

Pollinator observations

In total, plants were observed for 38 d (392.58 h were recorded), and 4 nights (44.83 h, Table 2). Flower observations revealed that only birds from Zosteropidae were effective pollinators of *A. bracteosum* (i.e., *Z. borbonicus*, the grey white-eye; Fig. 2A; 13 visits, and *Z. olivaceus*, the olive white-eye; Figs. 2B and 2C; 2 visits; Tables 2 and 3), even though flowers were also visited by a diurnal endemic species of hawkmoth (*Macroglossum milvus* Boisduval, Sphingidae, 2 visits), and by an unidentified species of fly (Diptera, 22 visits) during the day, and by a cortege of small arthropods at night, among which were spiders, centipedes, and a diversity of small insects (cockroaches, crickets, and moths other than *M. milvus*, Tables 2 and 3). However, the two species of bird did not pollinate the orchid with the same efficiency: if each visit of the olive white-eyes involved successful pollination events, only one visit of the grey white-eyes (out of 13) involved pollinarium removal or deposition (Tables 2 and 3). No difference in bird behaviour was observed: *Z. olivaceus* as well as *Z. borbonicus* typically landed on a leaf or inflorescence and probed the majority of “fresh looking” flowers.

Pollination and reproductive success at the population level

In PP, the flowering period lasted approximately from the beginning of February to the beginning of April, with a flowering peak at around the 10th of March. This phenology was constant throughout the 3 years. In BB, flowering period was approximately identical. Rates of pollen removal and deposition as well as fruit set are shown in Table 4, and statistics in Table 5.

Pollination and reproductive rates within a study site showed little variation between years, except for pollen deposition at PP that was significantly lower in 2004. The magnitude for all rates was higher in BB than in PP. However, this difference was only statistically significant for pollen removal in 2004 and for pollen deposition in 2005 (Table 5). Pollen deposition, pollen removal and fruit production were significantly correlated whatever the year or the population (pollen deposition – pollen removal: $R^2 = 0.30$, $p < 0.001$; pollen deposition – fruit production: $R^2 = 0.48$, $p < 0.001$; pollen removal – fruit production: $R^2 = 0.28$, $p < 0.001$; details per population and year not shown).

Concerning the different ecological factors that could affect pollination and fruiting success, four main hardwood native species were identified as hosts of *A. bracteosum* in

Table 1. In situ and ex situ fruit production according to the different pollination treatments.

	Number of fruits (<i>N</i> flowers; <i>N</i> plants)	Fruit set (%)
In situ		
Pollinator excluded	0 (38; 3)	0
Self-pollinations	37 (38; 2)	97.3
Cross-pollinations	35 (35; 5)	100
Ex situ		
Pollinator excluded	0 (50; 5)	0
Self-pollinations	4 (5; 5)	80.0
Cross-pollinations	6 (7; 5)	85.7

PP, in variable proportions: 45% of plants were found on *Philippia montana* Klotzch (Ericaceae), 35% on *Pandanus montanus* (Pandanaeae), 10% on *Geniostoma borbonicum* Spreng. (Loganiaceae), 5% on *Dombeya ficulnea* Baill. (Sterculiaceae), and 5% on other species. In BB, 67% of plants were found on *P. montana*, while 27% were found growing on the ground, 3% on *Nuxia verticillata* Lam. (Loganiaceae), and 3% on *Aphloia theiformis* (Vahl) Benn. (Flacourtiaceae). Host-tree diameter at the point of epiphyte placement varied from 18 to 60 cm (mean = 35.6 cm) in PP, and from 25 to 150 cm (mean = 48.0 cm) in BB. Epiphyte height on host tree ranged from 45 to 200 cm (mean = 136.8 cm) in PP, and from 0 to 200 cm (mean = 99.2 cm) in BB. These different factors (host species, host diameter, and epiphyte height) did not significantly influence pollen removal, pollen deposition, or fruit production, regardless of the year and the population sampled.

Discussion

Breeding system and nectar properties

Angraecum bracteosum is a fully self-compatible species, like all other species of subtribe Angraecinae for which reproductive strategy has been investigated (e.g., Nilsson et al. 1985, 1987; Nilsson and Rabakonandrianina 1988; Martins and Johnson 2007), but it requires pollinator services to achieve reproduction. Intensive hand pollinations of all flowers per inflorescence resulted in a high rate of fruit development, suggesting that reproductive success is strongly limited by pollinator activity, as is generally the case within Orchidaceae (see Tremblay et al. 2005 for more references). Although nectar of bird-pollinated species is in most cases abundant and dilute (Baker 1975; Bolten and Feinsinger 1978; Pyke and Waser 1981; Baker and Baker 1983; Nicolson 2002; Nicolson and Fleming 2003; Dupont et al. 2004; see also Micheneau et al. 2006), averaging (and ranging), respectively, 5.7 μL (2.7–11.7 μL) and 18.8% (9.7%–31.2%) sugar in sucrose equivalents (data from Rodríguez-Robles et al. 1992; Johnson 1996; Ellis and Johnson 1999; Singer and Sazima 2000; Johnson and Brown 2004; Micheneau et al. 2006), the nectar of *A. bracteosum* (4.9 μL ; 9.6% sugar in sucrose equivalents) is both less abundant and more dilute than the average volume and concentration reported for ornithophilous orchids. Of all sect. *Hadrangis* species, *A. bracteosum* is also the species that of-

Table 2. Details of videotape sessions: dates and recorded hours, number of observed flowers (N_{fl}), number of visits (N_{vi}), visitor and pollinator identification (bold indicates pollinators), and pollen movements (Pol mov^a) after a session.

Dates	Hours (total)	N_{fl}	N_{vi}	Visitor identification (h)	Pol mov ^a
Bébour DAY					
2005-Feb-16	0800 – 1100 (3 h)	27	0	—	No
2005-Feb-23	0700–1900 (12 h)	26	0	—	No
2005-Feb-24	0700–1300 (6 h)	26	0	—	No
Plaine des Palmistes DAY					
2005-Feb-22	0900–2100 (10 h)	5	0	—	No
2007-Feb-28	0615–1815 (12 h)	5	1	<i>Z. olivaceus</i> (11347)	Yes (2 Dep)
2007-Mar-1	0650–1845 (11.92 h)	7	2	<i>Z. borbonicus</i> (0832; 1652)	No
2007-Mar-2	0645–1845 (12 h)	8	0	—	No
2007-Mar-3	0640–1855 (12.25 h)	9	2	<i>Z. olivaceus</i> (0756) / <i>Z. borbonicus</i> (1457)	Yes (1 Rem)
2008-Feb-27	0820–1825 (9.92 h)	16	—	—	No
2008-Feb-27	0800–1815 (9.75 h)	27	3	<i>Z. borbonicus</i> (1312; 1512)– <i>M. milvus</i> (1813)	No
2008-Feb-28	0855–1800 (9.8 h)	15	1	<i>Z. borbonicus</i> (1607)	Yes (2 Rem)
2008-Feb-28	0900–1810 (9.17 h)	29	1	<i>Z. borbonicus</i> (1742)	No
2008-Mar-28	0705–1830 (11.33 h)	23	1	Diptera	No
2008-Mar-28	0720–1825 (11.8 h)	24	1	Diptera	No
2008-Mar-29	0700–1800 (11 h)	24	—	—	No
2008-Mar-29	0715–1810 (10.92 h55)	33	2	Diptera	No
2008-Mar-31	0800–1810 (10.17 h)	25	1	Diptera	No
2008-Mar-31	0810–1810 (10 h)	44	—	—	No
2008-Apr-1	0745–1710 (9.33 h)	39	—	—	No
2008-Apr-1	0720–1720 (10 h)	50	2	Diptera–Arachnida	No
2008-Apr-2	0715–1820 (11.8 h)	54	2	Diptera	No
2008-Apr-3	0705–1820 (11.25 h)	60	1	Diptera	No
2008-Apr-4	0655–1755 (11 h)	62	2	Diptera– <i>Z. borbonicus</i> (1521)	No
2008-Apr-5	0655–1805 (11.17 h)	67	2	Diptera	No
2008-Apr-6	0900–1810 (9.75 h)	67	—	—	No
2008-Apr-6	0800–1800 (9.67 h)	57	—	—	No
2008-Apr-7	0740–1735 (9.92 h)	69	2	Diptera	No
2008-Apr-7	0730–1730 (10 h)	44	3	Diptera	No
2008-Apr-8	0700–1810 (11.17 h)	71	3	Diptera	No
2008-Apr-9	0700–1815 (11.25 h)	71	1	<i>Z. borbonicus</i> (0910)	No
2008-Apr-10	0700–1810 (11.17 h)	74	1	<i>Z. borbonicus</i> (0749)	No
2008-Apr-11	0730–1745 (10.25 h)	74	1	<i>Z. borbonicus</i> (0941)	No
2008-Apr-14	0815–1805 (9.83 h)	78	1	<i>Z. borbonicus</i> (0849)	No
2008-Apr-14	0810–1810 (10 h)	34	2	Diptera– <i>M. milvus</i> (1300)	No
2008-Apr-15	0725–1825 (11 h)	78	—	—	No
2008-Apr-15	0720–1820 (11 h)	34	3	Diptera (×2)–Lepidoptera other than <i>M. milvus</i> (×1)	No
2008-Apr-16	0710–1810 (11 h)	81	1	<i>Z. borbonicus</i> (0748)	No
2008-Apr-16	0700–1800 (11 h)	34	2	Diptera	No
Plaine des Palmistes NIGHT					
2008-Mar-18	1900–0630 (11.50 h)	7	>20	Blattoptera–Chilopoda	No
2008-Mar-19	1850–0600 (11.17 h)	7	>20	Blattoptera–Chilopoda–Lepidoptera	No
2008-Mar-20	1850–0550 (11 h)	7	>20	Blattoptera–Chilopoda–Arachnida	No
2008-Mar-21	1950–0600 (11.17 h)	7	>20	Blattoptera–Chilopoda–Orthoptera (crickets)	No

Note: *Z.*, *Zosterops* (passerine bird); *M.*, *Macroglossum* (hawkmoth).

^aYes, if we observed either pollen deposition (Dep) or pollen removal (Rem) during a visit.

fers the least nectar to pollinators: nectar volume averaged 7.7 µL for *A. striatum* (Micheneau et al. 2006), and 16.7 µL for *A. cadetii* (Micheneau 2005). Sugar concentration, however, does not vary much among the three species: 9.6% in sucrose equivalents for *A. bracteosum*, and 9.7% for *A. striatum* and *A. cadetii* (Micheneau 2005; Micheneau et al. 2006).

Pollinator observations

White-eyes typically perched on inflorescences, and probed the majority of “fresh-looking” flowers for nectar. *Zosterops borbonicus* is extremely opportunistic in diet, and exploits a wide variety of flowers (both native and introduced, Table 6). It is therefore not surprising that this species, which pollinates *A. striatum* (Micheneau et al. 2006),

Fig. 2. Bird visitors of *Angraecum bracteosum* flowers. **A.** *Zosterops borbonicus* feeding on nectar. **B.** *Zosterops olivaceus* feeding on nectar. **C.** *Zosterops olivaceus* with pollinia attached on the tip of its beak (note the characteristic white-eye ring) (video-captures from C. Micheneau and J. Fournel, March 2007).

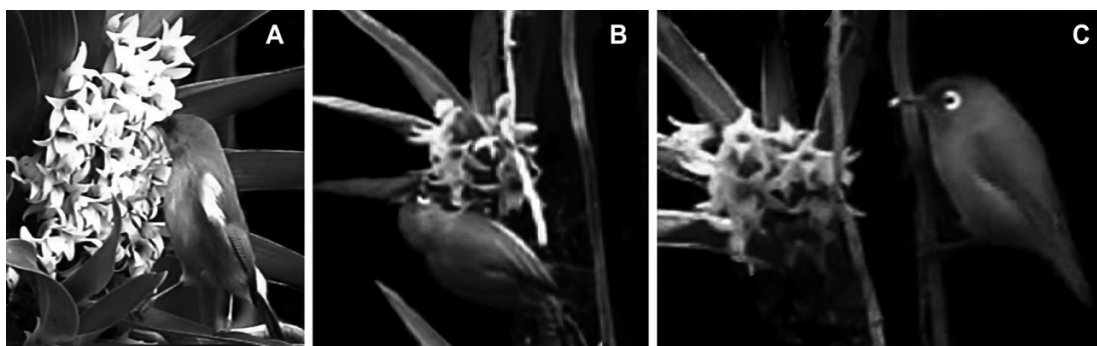


Table 3. Day and night flower visitors of *A. bracteosum*.

Flower visitor	Species	<i>N</i> visits	<i>N</i> visits/day or night	% Pollen movement
Day (<i>N</i> = 38)				
Bird	<i>Zosterops borbonicus</i>	13	0.34	7.7%
Bird	<i>Zosterops olivaceus</i>	2	0.05	100%
Hawkmoths (Sphingidae)	<i>Macroglossum milvus</i>	2	0.05	0%
Flies (Diptera)	Species 1	24	0.63	0%
Night (<i>N</i> = 4)				
Cockroaches (Blattoptera)		43	10.75	0%
Crickets (Orthoptera)		2	0.50	0%
Spiders (Arachnida)		1	0.25	0%
Centipedes (Chilopoda)		20	5	0%
Other moths (Lepidoptera)		1	0.25	0%

Note: Bold indicates pollinators. Pollen movement refers to pollen removal or deposition.

Table 4. Mean (\pm SD) values (percentages) of pollen removal, pollen deposition, and fruit set in two natural populations (BB and PP) of *A. bracteosum*, during 3 consecutive years (statistics are shown in Table 5).

Years	<i>N</i>	<i>N</i> _{fl}	Pollen removal (mean \pm SD)	Pollen deposition (mean \pm SD)	Fruit set (mean \pm SD)
BB					
2003	30	413	42.65 \pm 30.23	25.67 \pm 19.12	12.49 \pm 16.31
2004	28	403	47.97 \pm 27.51	18.66 \pm 26.31	9.00 \pm 15.69
2005	29	428	53.52 \pm 32.08	33.11 \pm 26.38	12.19 \pm 21.72
PP					
2003	20	428	39.40 \pm 26.94	21.59 \pm 14.25	5.98 \pm 6.45
2004	19	471	23.64 \pm 22.07	6.56 \pm 8.70	3.47 \pm 7.51
2005	17	417	26.21 \pm 34.17	10.44 \pm 13.56	5.74 \pm 10.33

Note: *N*: number of plants; *N*_{fl}: number of flowers.

and visits *A. cadetti* (the third species of sect. *Hadrangis*, C. Micheneau and J. Fournel, unpublished data, 2007), was also observed visiting flowers of *A. bracteosum*. However, it was more surprising that *Z. borbonicus* did not (or did rarely) remove pollinia when visiting flowers. If foraging by *Z. borbonicus* resulted in no (or few) transfers of pollinaria to *A. bracteosum* flowers, foraging by olive white-eyes resulted in a high frequency of pollinations (see Table 3). Olive white-eyes have unusually long and slender bills for white-eyes (averaging 15.6 mm in length, Gill 1971), which match perfectly with the narrow opening of the flower (aver-

aging 1.9 mm). In contrast, grey white-eyes display shorter and wider bills (averaging 3.7 mm in basal diameter, and 11.6 mm in length, Gill 1971; M. Lecorre, unpublished data, 2005), and it is likely that in most of the cases, grey white-eyes could not probe nectar on *A. bracteosum* flowers. Successful pollination events should occasionally be possible in unusually large flowers, or with unusually slender beaks. Spur length of *A. bracteosum* averages 8 mm in length, thus permitting the observed attachment of pollinia to the tip of a bird's beak (Fig. 2C), in contrast with the sister species, *A. striatum* (Micheneau et al. 2008), in which pollinia become attached to the opposite, basal end of the beak (Micheneau et al. 2006).

Zosterops olivaceus is believed to have colonized the Mascarene Archipelago before *Z. borbonicus* (Gill 1971; Warren et al. 2006). According to Gill (1971), the unusual beak morphology of *Z. olivaceus* is an adaptation to its almost exclusive nectar diet. The evolution of *Z. olivaceus* through nectarivory was possible on the Mascarenes, since no nectar-specialized birds are known to have been present on the islands before it was colonized by olive white-eyes: the ecological niche was available (Gill 1971). Gill (1971) explained that the subsequent competitive interaction following the colonization of the second white-eye species, *Z. borbonicus*, would have led "*Z. olivaceus* to become an even more specialized flower-feeder to shift from normal modes of insect foraging to fly-catching, and to give up fruit-eating", acquiring at the same time a more competitive and aggressive chasing behaviour for flower-feeding. *Angraecum bracteosum* is the only species of sect. *Hadrangis*

Table 5. χ^2 squared values from Kruskal–Wallis after correction for multiple comparisons using the sequential Sidak technique (Sokal and Rohlf 1995).

Factors	Variables	χ^2 values					
		Pollen removal		Pollen deposition		Fruit set	
BB	Year	1.96	NS	8.08	NS	0.67	NS
PP	Year	4.91	NS	13.8	***	1.66	NS
2003	Pop	0.15	NS	0.30	NS	1.12	NS
2004	Pop	8.46	**	3.20	NS	2.29	NS
2005	Pop	6.29	NS	9.37	**	0.34	NS

Note: NS, $p > 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table 6. Other plant species visited by *Zosterops olivaceus* (Zo) and *Z. borbonicus* (Zb) on Reunion.

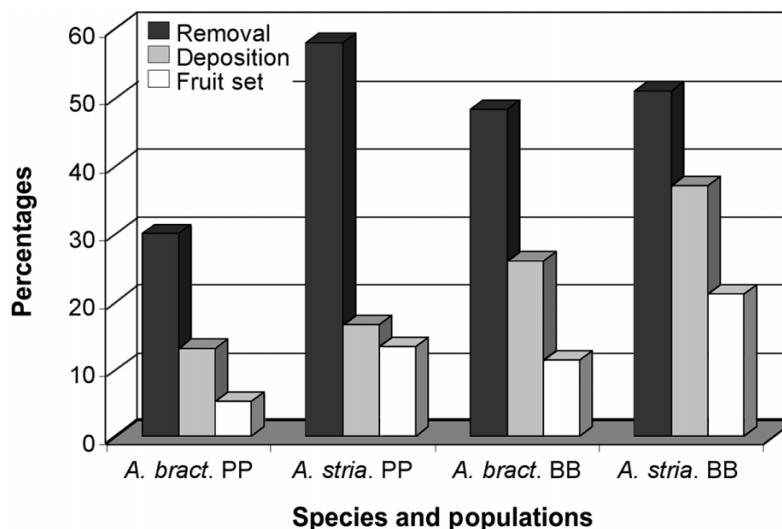
Species (Family)	Habit	Colour	Zo	Zb
Native plants				
<i>Angraecum striatum</i> (Orchidaceae)	Herb	White	?	x^a
<i>A. cadetii</i>	Herb	White	x^c	$x^{a,c}$
<i>Aphloia theiformis</i> (Flacourtiaceae)	Tree	White	x^b	x^a
<i>Dombeya elegans</i> (Sterculiaceae)	Shrub	Pink	x^c	x^a
<i>D. delislei–D. pilosa–D. reclinata</i>	Tree	White	x^c	x^a
<i>D. ficulnea–D. ciliata–D. punctata–D. blatiolens</i>	Tree	White–pink	x^c	x^a
<i>Eugenia buxifolia</i> (Myrtaceae)	Tree	White	?	x^a
<i>Forgesia racemosa</i> (Escaloniaceae)	Shrub	Red–pink	x^c	x^a
<i>Geniostoma borbonicum</i> (Loganiaceae)	Shrub	White	$x^{b,c}$	x^a
<i>Hibiscus boryanus</i> (Malvaceae)	Tree	Red	x^c	x^a
<i>Hypericum lanceolatum</i> (Hypericaceae)	Shrub	Yellow	$x^{b,c}$	x^a
<i>Lomatophyllum macrum</i> (Liliaceae)	Herb	Orange	?	x^a
<i>Molanea alternifolia</i> (Sapindaceae)	Tree	Cream	?	x^a
<i>Mussaenda landia</i> (Rubiaceae)	Tree	White	?	x^a
<i>Sophora denudata</i> (Fabaceae)	Tree	Yellow	$x^{b,c}$	x^a
<i>Syzigium borbonicum</i> (Myrtaceae)	Tree	White	?	x^a
<i>S. cordemoyi</i>	Tree	Red	?	x^a
<i>S. cymosum</i>	Tree	Red	x^c	x^a
<i>Trochetia granulata</i> (Sterculiaceae)	Shrub	White	?	x^a
Introduced plants				
<i>Callistemon citrinus</i> (Myrtaceae)	Tree	Red	?	x^a
<i>Camellia sinensis</i> (Theaceae)	Tree	Red	x^b	x^a
<i>Chrysalidocarpus lutescens</i> (Palmeaceae)	Tree	Cream	?	x^a
<i>Citrus</i> sp. (Rutaceae)	Shrub	White	x^c	?
<i>Cuphea platycentra</i> (Lythraceae)	Herb	Red	—	x^a
<i>Euphorbia pulcherrima</i> (Euphorbiaceae)	Shrub	Red	?	x^a
<i>Fuchsia magellanica</i> (Onagraceae)	Vine	Red	$x^{b,c}$	x^a
<i>Kalanchoe pinnata</i> . (Crassulaceae)	Herb	Cream	x^b	x^a
<i>Lantana camara</i> (Verbenaceae)	Shrub	Yellow to Pink	x^b	x^a
<i>Lomatophyllum purpureum</i> (Liliaceae)	Herb	Red	?	x^a
<i>L. tormentorii</i> (Liliaceae)	Herb	Orange	?	x^a
<i>Melia azedarach</i> (Meliaceae)	Tree	White–Violet	x^b	?
<i>Musa acuminata</i> (Musaceae)	Shrub	Cream	x^b	x^a
<i>Pelargonium capitalum</i> (Geraniaceae)	Herb	Pink to Violet	x^b	?
<i>Rubus alceifolius</i> (Rosaceae)	Vine	White	?	x^a
<i>Spathodea campanulata</i> (Bignoniaceae)	Tree	Orange	?	x^a
<i>Syzigium jambos</i> (Myrtaceae)	Tree	White–Pink	$x^{b,c}$	x^a

^aMicheneau et al. 2006.

^bGill 1971.

^cAuthors, personal observations.

Fig. 3. Comparison of pollination and fruiting success between *Angraecum bracteosum* (*A. bract.*) and *A. striatum* (*A. stria.*) in both study sites: La Plaine des Palmistes (PP) and Bébouir (BB). Means of the three years are presented. Sample sizes for *A. striatum* (i) in 2003: BB, 325 flowers/34 individuals were studied; PP, 303 flowers/28 individuals; (ii) in 2004: BB, 299 flowers/33 individuals; PP, 100 flowers/21 individuals; (iii) in 2005: BB, 422 flowers/35 individuals; PP, 164 flowers/16 individuals.



gis having a strong fold on the lip, a convergent feature often found in bird-pollinated orchids (e.g., van der Pijl and Dodson 1966; van der Cingel 2001), and revealing a high degree of bird pollination adaptation. However, the sequence of evolutionary events leading to the establishment of *Zosterops-Hadrangis* interactions in the Mascarene remains undetermined.

Pollination and fruiting success

At the BB study site, and during three consecutive years, pollen removal rates ranged approximately from 43% to 54%, while pollen deposition ranged from 19% to 33%. At the PP site, pollination success was a little bit lower, ranging from 24% to 39% for pollen removal (significant in 2004) and from 7% to 22% for pollen deposition (significant in 2005). The BB site was situated in the heart of one of the best preserved mountain cloud forests of Reunion, while the PP site was situated on the edge of the forest, close to urban areas. The effect of differences in the quality of sites (in terms of preservation and integrity of habitats), on bird population sizes could explain why pollination success was better in BB than in PP. Fruit set followed the same pattern, ranging from 9% to 12% in BB, and from 3% to 6% in PP. No significant correlation between pollination and reproductive success or between pollination and epiphyte height was found in PP or BB, suggesting that birds take nectar even on plants that are growing on the ground, and therefore that ground-dwelling is not disadvantageous in terms of reproduction. Birds were active within the whole population of *A. bracteosum*, as 90%–93% of sampled individuals had at least one flower with its pollinarium removed in BB and 76.5%–95% in PP.

In comparison with the sister species, *A. striatum* (Micheneau et al. 2008), pollination and fruiting success of *A. bracteosum* were always inferior at both sites (Fig. 3). On Reunion, *Z. borbonicus* is one of the most common passerine birds (465 000 individuals were estimated in 1983, Barau et al. 2005), occurring over the whole island from sea

level to 2300 m, with a large habitat range including both primary forests and inhabited disturbed human zones. In contrast, *Z. olivaceus* is less common on the island (150 000 individuals are estimated, Barau et al. 2005), and is found in preserved habitats from 500 m a.s.l. (rarely 200 m a.s.l.) to 2400 m a.s.l., only. If *Z. borbonicus* visited *A. bracteosum*'s flowers more often than *Z. olivaceus*, its foraging involved few pollinations, contrary to its foraging on *A. striatum*'s flowers, which involved high levels of pollen removal and deposition (Micheneau et al. 2006). Plants of *A. striatum* display larger flowers, with a wider opening (averaging 4.1 mm).

In comparison with other bird-pollinated orchids, reproductive success of *A. bracteosum* (i.e., approximately 11% in PP and 15% in BB) is the lowest reported in the literature: for example, fruit set ranges from 18% for the tropical epiphytic hummingbird-pollinated *Compartmentia falcata* (Rodríguez-Robles et al. 1992) to 95% for the temperate ground-dwelling sunbird-pollinated *Disa chrysostachya* (Johnson and Brown 2004).

Concluding remarks

This study provides a novel example of bird-pollination within the Mascarene endemic *Angraecum* sect. *Hadrangis*. Although *A. striatum* is pollinated by the grey white-eyes, its sister species, *A. bracteosum*, is predominantly pollinated by the olive white-eyes, the second species of *Zosterops* occurring on Reunion. This study highlighted a probable case of pollinator specialization within *Angraecum* sect. *Hadrangis*. It remains to be determined whether pollinator-mediated selection has driven the divergence between these two sister orchid species (i.e., sympatric speciation) on Reunion.

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