

INVITED REVIEW

Orchid pollination: from Darwin to the present day

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In this year celebrating the bicentenary of the birth of Darwin and the sesquicentennial of the publication of Darwin's *On the Origin of Species*, the present paper aims to assess the impact of Darwin's legacy on the history of orchid pollination biology. To illustrate the major contribution of Darwin to this fascinating biological field, we focus on the large angraecoid orchid group and propose an overview of the complex relationships that these orchids have developed with specific pollinators. We further discuss how Darwin's seminal work on the angraecoid orchid *Angraecum sesquipedale* triggered the beginning of a long debate about the evolution of long floral spurs and why his idea of reciprocal evolution or 'coevolution' was one of the great contributions to evolutionary biology. © 2009 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2009, **161**, 1–19.

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INTRODUCTION

For the celebration of the bicentenary of Charles Darwin's birth and the sesquicentennial of the publication of his *On the Origin of Species* (Darwin, 1859), the world is re-exploring the impact of Darwin and his theory of evolution on science and society. However, it is not possible to commemorate Darwin's legacy without consideration of orchids, which took a central place in his main achievements, and to which he devoted many years of study: 'I never was more interested in my life in any subject than this of orchids' (Darwin, 1861 in a letter addressed to Hooker).

The subject of the present paper is to revisit the history of orchid pollination biology from the time of Darwin to the present day, with an emphasis on the

pollination mechanisms involved in the angraecoid orchids, including the famous long-spurred Madagascan species, which, through Darwin's meticulous observations and innovative reasoning, served to bring the orchid family into the heart of evolutionary research on plant–pollinator interactions.

ORCHID POLLINATION: A BRIEF HISTORY

FROM DARWIN'S TIME TO THE 1930S

Although, by the end of the 18th century, earlier botanists including Bernard de Jussieu and Michel Adanson had contributed to the better understanding of the general structure of orchid flowers, pollination mechanisms in orchids were first fully detailed by Darwin in his famous book, *On the various contrivances by which British and foreign orchids are fertilised by insects and the good effects of intercrossing* (Darwin, 1862). In addition to being 'an inspiration to floral ecologists of his and later time' (van der Pijl & Dodson, 1966), Darwin's book remains a classic today.

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In this book, he described his own observations on the pollination of many British orchids, his personal interpretations of the pollination mechanisms involved in some tropical species, mainly based on floral morphology, and his views on homologies in orchid flowers. The second edition, published in 1877, included an extended part dedicated to tropical orchids, based mainly on the observations made by his contemporary Fritz Müller (Darwin, 1877). These two books, in which Darwin clearly demonstrated that orchids provide strong evidence for natural selection and for adaptations that promote cross-pollination, were written partly in support of his theory of evolution, which invoked much criticism after the publication of *On the Origin of Species* in 1859 (Darwin, 1859). However, Darwin's idea that orchids provide evidence for adaptations to insect pollination found strong support among naturalists and influenced as never before the future directions of the fascinating field of orchid pollination biology. From that time until the 1920s, the Western world raced to gather information on insect–flower interactions, involving ‘most of our knowledge on the pollination mechanisms and insect visitors of European and North American flowers’ (Proctor, Yeo & Lack, 1996).

The next major contribution to orchid pollination had to wait for the end of this descriptive period and the discovery of pseudocopulation in the genus *Ophrys* L. in Algeria (Correvon & Pouyanne, 1916; Pouyanne, 1917) and, soon after, in the genus *Cryptostylis* R.Br. in south-eastern Australia (Coleman, 1927). Sexual deception in flowers which mimic the signals of female insects, in some cases so accurately that they elicit copulatory behaviour in male insects (pseudocopulation), is almost exclusive to orchids and constitutes one of the most fascinating pollination mechanisms found in the family, if not in all flowering plants. Male insects are sexually attracted to the flower by a floral scent that imitates the olfactory cue or sex pheromone(s) of the female, often enhanced with a resemblance to the female in shape, colour and texture, and usually pick up and deposit pollen while they attempt copulation with flowers. Since its discovery in the 1920s, sexual deception has been found in a number of other orchids and remains an active topic of interest in orchid pollination and evolution (e.g. Schiestl *et al.*, 1999, 2003; Ayasse *et al.*, 2002; Singer, 2002; Singer *et al.*, 2004).

FROM THE 1930S TO THE 1960S

During the next three decades, interest in orchid floral biology and pollinator observations declined, as the attention of biologists shifted to new disciplines such as cytology and genetics. It was only in 1966

that the second classic book of orchid pollination, *Orchid flowers, their pollination and evolution*, was published to commemorate the centenary of the publication of Darwin's book (van der Pijl & Dodson, 1966). In addition to being a review of one century of orchid–pollinator observations, this book constituted a major update, taking advantage of recent observations by the authors themselves and contributions provided by other famous orchidologists including R. L. Dressler and B. Kullenberg. Van der Pijl and Dodson presented a modern appreciation of orchid pollination syndromes with an emphasis of the major role played by pollinators in the evolutionary history of the orchid family. In this, their views were deeply rooted in Darwinian thought.

SINCE THE 1960S

From the end of the 1960s, there was a resurgence of interest in pollination studies. This can be attributed to the development of ecology and new evolutionary concepts and models, many of which were integrated into the field known at that time as ‘biosystematics’ (Proctor *et al.* 1996). In addition, the formalization of new principles and methods (Jones & Little, 1983; Willson, 1983; Dafni, 1992; Kearns & Inouye, 1993) contributed to a redefinition of the scope of pollination studies as an integrated part of broader studies of reproductive biology and population dynamics. Especially in orchids, in which pollinator activity could be easily quantified by pollinarium removal and deposition, an incredible volume of quantitative data on pollination and reproductive success, both in time and space, has been gathered throughout the world: in Europe and North America (e.g. Nilsson, 1981, 1983a; Gigord, Macnair & Smithson, 2001; Smithson & Gigord, 2001; Smithson, 2002), the Neotropics and Australia (e.g. Romero & Nelson, 1986; Peakall, Beattie & James, 1987; Peakall, 1989; Zimmerman & Aide, 1989; Ackerman & Montalvo, 1990; Rodríguez-Robles, Meléndez & Ackerman, 1992; Ackerman, Rodríguez-Robles & Meléndez, 1994; Ackerman, Meléndez-Ackerman & Salguero-Faria, 1997; Tremblay *et al.*, 1998; Borba & Semir, 1999; Borba, Shepherd & Semir, 1999; Salguero-Faria & Ackerman, 1999; Singer & Cocucci, 1999; Parra-Tabla *et al.*, 2000; Borba & Semir, 2001; Singer, 2001; Borba *et al.*, 2002; Singer, 2002; Schiestl *et al.*, 2003; Singer *et al.*, 2004) and in South Africa (Steiner, Witthead & Johnson, 1994; Johnson & Steiner, 1997; Johnson, 1997a, b; Johnson & Kurzweil, 1998; Johnson, Linder & Steiner, 1998; Ellis & Johnson, 1999). Although noteworthy books and reviews contributed to enhancing our knowledge of the identity and specificity of orchid pollinators and their role in orchid evolution (e.g. Ackerman, 1986; Nilsson, 1992; Tremblay, 1992;

van der Cingel, 1995; Johnson & Steiner, 2000; van der Cingel, 2001; see also Dressler, 1993), three publications, in particular, have synthesized the main conclusions that arose from this productive period. Firstly, Neiland and Wilcock (1998) undertook a comparative study of the reproductive success among 117 nectariferous and nectarless orchids, from both temperate and tropical regions. They confirmed the hypothesis that nectariferous orchids are more successful in setting fruit than nectarless species, but they also expressed concern about the lack of data on reproductive success in the tropical regions. Secondly, Tremblay *et al.* (2005) presented an extensive overview of orchid floral biology and reproductive success and their evolutionary consequences for orchid diversification. They reaffirmed that the seed production in representatives of the family is frequently strongly pollinator-limited and suggested that genetic drift, related to the small number of individuals that participate in reproduction when there is pollen limitation, could have played a role in the extraordinary diversification of the family. Thirdly, Jersáková, Johnson & Kindlmann (2006) reviewed the mechanisms of deceptive pollination in the orchid family and the various hypotheses that have been put forward to account for their evolution. They concluded that promotion of cross-pollination was the most compelling argument for the evolutionary maintenance of floral deception in about 40% of species in the family.

In addition, the combined development of molecular biology (DNA sequencing) and phylogenetic methods during the last two decades opened a vast new field of investigation, allowing the scientific community to revisit hypotheses about relationships among organisms. The mapping of pollination syndromes (or pollinator-linked floral traits) onto phylogenetic trees has provided new insights into the ecological determination of evolutionary transitions, their polarity, the extent to which changes were evolutionarily labile or constrained and the extent to which radiations can be explained by key trait innovations (e.g. Larsen *et al.*, 2008). A comprehensive phylogeny of the orchid family based on molecular data was lacking until the contribution of Chase *et al.* (2003) and such phylogenies have been crucial in determining evolutionary transitions, especially for orchids in which extensive floral convergence as a result of pollinator-mediated selection has occurred.

Recently, Ramírez *et al.* (2007) estimated ages for the origin of the orchid family and each subfamily based on pollinia found fossilized in amber. We can now explore how orchid–pollinator interactions developed over time, which may allow us to distinguish between coevolution (plants and pollinators evolve together reciprocally) vs. sequential evolution (when the evolution of one group follows another).

Pollination remains an active field of research and papers are still being published in large numbers as new data are accumulated. Some groups of orchids have been and still are the focus of study, including various members of the largest subfamily, Epidendroideae (e.g. Stpiczyńska, Davies & Gregg, 2007; Brys, Jacquemyn & Hermy, 2008; Chase *et al.*, 2009; Pansarin, de Moraes Castro & Sazima, 2009), the bee orchids and their allies (*Ophrys* L., Orchidoideae, e.g. Cortis *et al.*, 2009; Devey *et al.*, 2009; Schlüter *et al.*, 2009) and the lady's slipper orchids (species of *Cypripedium* L., Cypridioideae, e.g. Bänziger, Sun & Luo, 2008; Case & Bradford, 2009; Li *et al.*, 2008), for which an elegant new method for studying pollination was recently published by Case & Bradford (2009). In a recent special issue of *Annals of Botany* focusing on orchid biology (Fay & Chase, 2009, and associated papers), pollination of orchids was discussed in several papers. In addition to two reporting pollination studies (Cheng *et al.*, 2009; Peter & Johnson, 2009), these discussed pollination in relation to taxonomy and phylogenetic relationships (Chase *et al.*, 2009; Hopper, 2009; Salazar *et al.*, 2009; van den Berg *et al.*, 2009), species delimitation (Cortis *et al.*, 2009; Devey *et al.*, 2009; Hedrén & Nordström, 2009), inbreeding vs. outbreeding (Duffy *et al.*, 2009), invasiveness (Cohen & Ackerman, 2009), conservation (Swarts & Dixon, 2009) and evolutionary development (Mondragón-Palomino & Theißen, 2009).

CASE STUDY: ANGRAECOID ORCHID POLLINATION

BACKGROUND

The angraecoid orchids (Epidendroideae Lindl., Vandeeae Lindl., Angraecinae Summerh. *sensu lato*; see below) are a large group of monopodial epiphytic orchids, including more than 760 species. Two genera have diversified in Central and South America and the Caribbean (*Campylocentrum* Benth. and *Dendrophylax* Rchb.f.), but most of these orchids are the product of an extensive radiation in the western Indian Ocean, including Africa, Madagascar and nearby islands. Although angraecoid orchids were historically referred to two subtribes, Angraecinae (c. 17 genera, 410 species, mostly from Madagascar) and Aerangidinae Summerh. (c. 32 genera, 350 species, mostly from Africa) (e.g. Schlechter, 1926; Dressler, 1981, 1993; Chase *et al.*, 2003), recent insights from molecular data provided evidence to support recognition of a single subtribe, Angraecinae, which includes all angraecoid orchids (i.e. Angraecinae *sensu lato*; Carlswald *et al.*, 2006).

The Old World angraecoid orchids are known for their white, spectacularly long-spurred flowers highly

specialized for hawkmoth (sphingid) pollination. The study of the pollination biology of these orchids started with the famous prediction by Darwin, which, in spite of being widely known, deserves to be repeated here. Darwin received some specimens of *Angraecum sesquipedale* Thou. and was particularly intrigued by the peculiar flowers of this strange orchid which, for the plants he examined, had a nectar tube approximately '11 and a half inches long' (almost 30 cm). After meticulous floral observations and different experiments of manual pollination, he concluded that only a giant hawkmoth with a 'wonderfully long proboscis' would be able to pollinate this spectacular orchid. The predicted pollinator, *Xanthopan morganii* var. *praedicta*, was found 41 years later in the primary forests of Madagascar (Rothschild & Jordan, 1903), but effective pollination of the orchid by this animal was only demonstrated 135 years after Darwin's prediction (Wasserthal, 1997)! In addition to predicting the existence of a giant hawkmoth on Madagascar, Darwin suggested that the extraordinary length of the spur of *A. sesquipedale* was driven by natural selection because of an advantage for reproduction success when the spur is longer than the moth proboscis and the head of the moth presses firmly against the orchid column, enhancing pollination. Conversely, if the orchid spur was longer than the proboscis, selection would favour a longer proboscis because this would enable the moth to gain access to more nectar. Darwin thus posited that there would be a kind of arms race between the spur length of the orchid and the proboscis of the moth (see Nilsson, 1988; Anderson & Johnson, 2008; Pauw, Stofberg & Waterman, 2009). This idea of reciprocal evolution or 'coevolution' was one of Darwin's great contributions to evolutionary biology.

Since this famous prediction, the large genus *Angraecum* Bory has received much attention from evolutionary biologists (e.g. Wallace, 1867, 1871; Nilsson, 1992, 1998b; Wasserthal, 1997). However, floral morphology is diverse within the genus, and in the subtribe, suggesting variation in pollination systems. Notably on the Mascarene islands (Mauritius, Reunion and Rodriguez, Indian Ocean), three species (*Angraecum striatum* Thou., *A. bracteosum* Balf.f. & S.Moore and *A. cadetii* Bosser) are particularly interesting because their white flowers have short, wide spurs. These features were sufficiently uncommon in the genus to prompt Bosser (1987) to revise section *Hadrangis* Schltr. to include only these three species, making the section endemic to the Mascarenes. Pollinator observations have shown that two of these species are pollinated by small songbirds from the family Zosteropidae (white-eyes) on Reunion (Micheneau, Fournel & Paillet, 2006; Micheneau *et al.*, 2008c), whereas the third species, *A. cadetii*

(Reunion and Mauritius) has a highly surprising pollinator, a raspy cricket (Gryllacrididae from the order Orthoptera) (Micheneau *et al.*, in press). At the opposite end of the scale, all endemic long-spurred species are self-pollinated on Reunion (Micheneau, 2005; Micheneau *et al.*, 2008b; see also Jacquemyn *et al.*, 2005). These evolutionary shifts (from outcrossing to selfing and between different pollinators) are related to recent dispersal to the Mascarene Islands, where specific long-tongue pollinators of ancestral orchid colonists were probably absent. From combined phylogenetic, biogeographic and ecological data, it now appears that angraecoid orchid diversification was strongly associated with interactions with the pollinator fauna on Reunion (Micheneau *et al.*, 2008a). In contrast, self-pollinating species appear less likely to be involved in adaptive radiations. In the following, we survey the current state of knowledge of the pollination of angraecoid orchids.

METHODS INVOLVED IN POLLINATOR OBSERVATION

Various methods have been used to observe pollinators foraging on angraecoid orchid flowers and these are representative of methods that may be used to observe pollinator activity on plants more generally (see also Kearns & Inouye, 1993). Pollinators may be identified directly in the field by observing their foraging on flowers. Direct observations are commonly undertaken in a time period that corresponds to the main activity of the expected pollinators (i.e. the evening in the case of hawkmoth pollinators, e.g. Martins & Johnson, 2007). However direct observations may be time-consuming, particularly if there are low visitation rates or if pollinator behaviour is altered by human presence (e.g. birds). Depending on the behaviour of pollinators, direct observations may also involve difficulties in distinguishing simple visitors from efficient pollinators. Videotape observations in the field offer a good alternative for observing pollinator visits without influencing their behaviour and without spending days in front of flowers while nothing is happening. The development of hard-disk camcorders with night option, combined with the use of long-duration rechargeable batteries and a waterproof casing, now allow the observation of pollinators for more than 12 h, whatever the time of day or night and whatever the weather (Micheneau *et al.*, 2006, 2008c, in press). It is also possible to identify pollinators using indirect techniques: identifiable pollinaria may be found on pollinators caught on flowers of non-orchid species or at a light trap (e.g. Dressler, 1981; Nilsson *et al.*, 1985, 1987; Luyt & Johnson, 2001) and identifiable insect scales and hairs may be found on orchid stigmas (e.g. Nilsson & Rabakonandrianina, 1988). In these cases, pollination

mechanisms can be deduced on the basis of floral morphology and the attachment of pollinaria on the body of the pollinators, even if observations have been largely unsuccessful in the field (e.g. Nilsson & Rabakonandrianina, 1988; Luyt & Johnson, 2001).

BREEDING SYSTEMS

All angraecoid orchids that have been studied are self-compatible (Nilsson & Rabakonandrianina, 1988; Luyt & Johnson, 2001; Micheneau, 2005; Micheneau *et al.*, 2006, 2008b,c; Martins & Johnson, 2007; see also Dressler, 1993). In almost all cases, fruit set is not significantly different between self- and cross-pollinated flowers. However, in *Mystacidium venosum* Harv. ex Rolfe, self-pollination significantly decreased fruit weight, fruit length and the percentage of seeds with embryos, which could reflect either partial self-incompatibility or, more likely, strong inbreeding depression (Luyt & Johnson, 2001). These patterns are like those observed at the family level: self-compatibility, as a result of the capacity to set fruits from self-pollen is largely dominant within Orchidaceae (self-incompatible is estimated to occur in at least 10% of all species; Dressler, 1993), resulting in no difference in the number of fruits between selfed and crossed treatments (Tremblay *et al.*, 2005). However, self-pollination, via a significant decrease in embryo formation in mature capsules, tends to affect seed production (see Darwin, 1875; Tremblay *et al.*, 2005). True self-incompatibility is known from just a few orchids. Darwin stated in the second edition of *The variation of animals and plants under domestication* (1875): 'As these orchids had been grown under unnatural conditions in hot-houses, I concluded that their self-sterility was due to this cause. But Fritz Müller informs me that at Desterro, in Brazil, he fertilised above one hundred flowers of the above-mentioned *Oncidium flexuosum* Lodd., which is there endemic, with its own pollen and with that taken from distinct plants: all the former were sterile, whilst those fertilised by pollen from any other plant of the same species were fertile'. [For a discussion of this and other examples of the extensive correspondence between Darwin and Müller, see West (2003)]. According to Tremblay *et al.* (2005), 'Given the apparent costs of self-pollination in most orchids, mechanisms that promote outcrossing are to be expected. What is unusual is that in orchids these are often structural (movement of rostellum, movement of column, stigmatic maturity) and not the result of genetic self-incompatibility, dichogamy, or unisexuality (although examples of all of these mechanisms exist in orchids)', which led them to address an open question for future research: 'is it the very existence of pollinaria and column that has promoted the reliance of orchids on structural mechanisms that promote outcrossing?'

Although all angraecoid orchids studied so far are self-compatible, most, but not all, require pollinator services to achieve reproduction. A notable exception to this general rule is found on the Mascarenes, where the six long-spurred species endemic to the archipelago are all able to set fruit without pollen vectors (Jacquemyn *et al.*, 2005; Micheneau, 2005; Micheneau *et al.*, 2008b). Self-pollination was not expected within the angraecoid orchids, which, as mentioned before, have long been considered as a highly moth-pollinator-dependent group. Within the tribe, Dressler (1993) estimated the number of self-pollinating vanda species as <1%, whereas estimates of self-pollination in Orchidaceae as a whole range between 5 and 20% of species (Catling, 1990). However, high rates of self-pollination are not surprising in island floras; shifts to self-pollination are thought to provide reproductive assurance when the pollinator fauna is lacking, sparse or unspecialized (Ackerman, 1985; Barrett, 1985; Catling, 1990; Barrett, 1996; Eckert, Samis & Dart, 2006). On the isolated volcanic island of Reunion, the proportion of self-pollinating orchids is however, among the highest in the world, estimated at 46% of species (i.e. 56 out of 121 species of orchids; Jacquemyn *et al.*, 2005). To date, only the boreal flora of the northern hemisphere has been reported to include a greater proportion of self-pollinated species (c. 50% of c. 40 species; Catling, 1990). On Reunion, the distribution of self-pollinating orchids is significantly correlated with the high elevational gradient found on the island (c. 2500 m; Jacquemyn *et al.*, 2005). However, angraecoid orchids do not follow this pattern; self-pollination in these species is strongly positively correlated with spur length (Fig. 1), suggesting that species specialized for pollination by long-tongued hawkmoths underwent selection for self-pollination because these moths were absent or scarce at the time of colonization.

POLLINATOR IDENTITY AND SPECIFICITY WITHIN THE ANGRAECOID ORCHIDS

Pollinator identity

A list of published pollinators of angraecoid orchid species is shown in Table 1. These records include long-tongued sphingids from Madagascar and Africa, white-eye species from the Mascarenes and insular orthopterans (see also Fig. 2). As expected, the two famous orchid species with the longest spurs (often >30 cm in length), *Angraecum sesquipedale* and *A. sororium* Schltr., are pollinated by the two sphingid species that possess the longest tongues in Madagascar (often >20 cm), *Xanthopan morgani* var. *praedicta* and *Coelonia solani*, respectively (Wasserthal, 1997; Table 1). However, although the hawkmoth fauna of Madagascar (c. 60 species) is highly endemic and

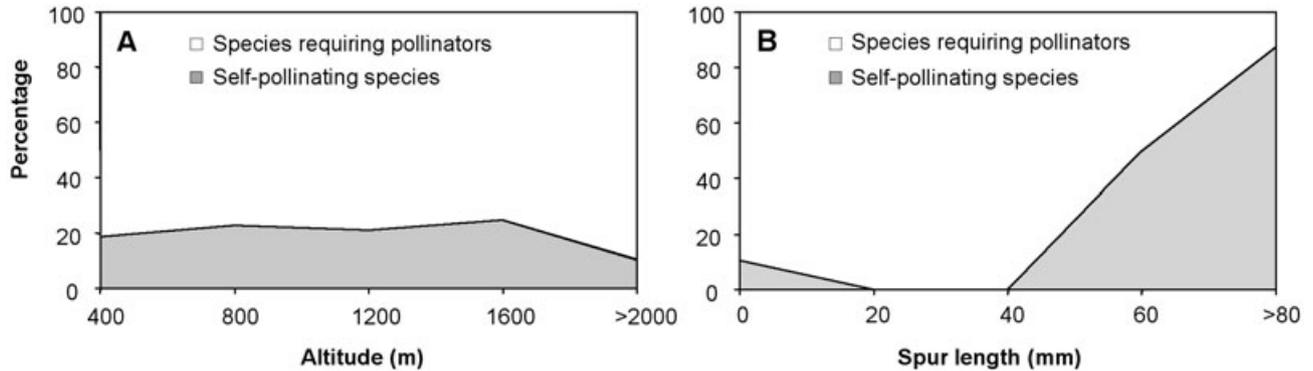


Figure 1. Distribution of self-pollinated angraecoid orchids on Reunion according to (A) altitude and (B) spur length (data from Jacquemyn *et al.*, 2005; Micheneau, 2005).

Table 1. Checklist of published pollinators of angraecoid orchids with the place of the attachment of pollinaria on the body of the pollinator (S, sphingids; B, birds; O, orthopterans)

Orchid species	Attachment of pollinarium
Spur length > 20 cm (Madagascar)	
<i>Angraecum sesquipedale</i> †	<i>Xanthopan morgani</i> (S) Base of the proboscis
<i>Angraecum sororium</i> †	<i>Coelonia solani</i> (S) Base of the proboscis
Spur length < 20 and > 10 cm (Madagascar, Kenya)	
<i>Angraecum arachnites</i> *§	<i>Panogena lingens</i> (S) Base of the proboscis ventrally
<i>Angraecum compactum</i> †§	<i>Xanthopan morgani</i> , <i>Panogena lingens</i> (S) Base of the proboscis dorsally
<i>Aerangis ellisii</i> ‡	<i>Agrius convolvuli</i> , <i>Panogena lingens</i> (S) N/A¶¶
<i>Neobathia grandidierana</i> §	<i>Panogena lingens</i> (S) Base of the proboscis dorsally
<i>Jumellea teretifolia</i> §	<i>Panogena lingens</i> (S) Base of the proboscis dorsally
<i>Aerangis fuscata</i> §	<i>Panogena lingens</i> (S) Frons or palps of the head
<i>Rangaeria amaniensis</i> ¶	<i>Agrius convolvuli</i> (S) Head or base of the proboscis¶¶
<i>Aerangis brachycarpa</i> ¶	<i>Agrius convolvuli</i> , <i>Coelonia fulvinotata</i> (S) Base at the proboscis dorsally
<i>Aerangis thomsonii</i> ¶	<i>Coelonia fulvinotata</i> (S) Head or base of the proboscis***
Spur length c. 5 cm (Kenya, South Africa)	
<i>Mystacidium venosum</i> **	<i>Nephele accentifera accentifera</i> (S) Base of the proboscis dorsally
<i>Aerangis confusa</i> ¶	<i>Hippotion celerio</i> , <i>Daphnis nerii</i> (S) Base of the proboscis dorsally
Spur length < 2 cm (Reunion)	
<i>Angraecum striatum</i> ††	<i>Zosterops borbonicus</i> (B) Base of the beak
<i>Angraecum bracteosum</i> ‡‡	<i>Zosterops olivaceus</i> , <i>Z. borbonicus</i> (rare) (B) Tip of the beak
<i>Angraecum cadetii</i> §§	<i>Glomeremus</i> sp. (O) Top of the head

*Nilsson *et al.* (1985).

†Wasserthal (1997).

‡Nilsson & Rabakonandrianina (1988).

§Nilsson *et al.* (1987).

¶Martins & Johnson (2007).

**Luyt & Johnson (2001).

††Micheneau *et al.* (2006).

‡‡Micheneau *et al.* (2008c).

§§Micheneau *et al.* (in press).

¶¶Pollinators were identified from hairs found on orchid stigmas.

***Not clearly precised by the authors.

N/A: Not applicable.



Figure 2. Illustrations of different pollinator groups. A, *Hippotion celerio* visiting flowers of *Mystacidium capense* (L.f.) Schltr. (South Africa, picture from S. D. Johnson). B, C, *Angraecum striatum* and its bird-pollinator *Zosterops borbonicus* (grey white-eyes) with pollinaria attached on its beak (pictures taken from videocaptures, Reunion, C. Micheneau and J. Fournel). D, *Angraecum cadetii* and its raspy cricket-pollinator *Glomeremus* sp. with pollinaria attached on its head (pictures taken from videocaptures with night-shot option, Reunion, C. Micheneau and J. Fournel). E, Illustration of a whole plant of *A. cadetii* with a visit of the grey white-eyes (note that the bird is a simple flower visitor and does not pollinate this orchid species; pictures taken from videocaptures, Reunion, C. Micheneau and J. Fournel).

comparatively rich in long-tongued taxa, one species, *Panogena lingens*, is by far the most common pollinator of the Madagascan angraecoid species and the sole pollen vector of at least four orchid species (Table 1). This hawkmoth is widely distributed in Madagascar and shows a bimodal distribution in tongue length and width. Two distinct morphs have been recognized, the long- and slender-tongued morph with a mean proboscis length of *c.* 11–12 cm and the shorter-tongued morph with a mean proboscis length of *c.* 7–8 cm. The long-tongued morphs were often the only or most effective pollinators (Nilsson *et al.*, 1987; Nilsson & Rabakonandrianina, 1988; see however, Wasserthal, 1997). In Africa, *Agrius convolvuli* and *Coelonia fulviventata* were the two most common pollinators of *Aerangis* Rchb.f. and *Rangaeris* (Schltr.) Summerh. species (Fig. 2A, Martins & Johnson, 2007). On the island of Reunion, our studies conducted on the short-spurred angraecoid orchids, *Angraecum striatum* and *A. bracteosum*, revealed that the two white-eye species that occur on the island, the grey white-eye, *Zosterops borbonicus*, and the olive white-eye, *Z. olivaceus*, were both implicated in the pollination of these orchids: grey white-eyes are the regular pollinators of *A. striatum* and occasionally pollinate *A. bracteosum* and olive white-eyes are the regular pollinators of *A. bracteosum*, but have never been observed foraging on *A. striatum* flowers (Fig. 2; Micheneau *et al.*, 2006, 2008c). Although around 50 bird families have been reported as flower visitors (Proctor *et al.* 1996), three major avian radiations have independently evolved a specialized nectar diet: hummingbirds (Trochilidae) in the New World, sunbirds (Nectariniidae) in the Old World (primarily Africa) and honeyeaters (Meliphagidae) in Australasia (e.g. van der Pijl & Dodson, 1966; Wolf, Hainsworth & Gill., 1975; Stiles, 1981; Proctor *et al.*, 1996; Anderson, 2003). Although these three groups of birds have been widely reported as orchid pollinators (e.g. van der Pijl & Dodson, 1966; Rodríguez-Robles *et al.*, 1992; Johnson, 1996; Singer & Sazima, 2000; Johnson & Brown, 2004), pollination of orchids by birds outside these three main nectarivorous groups (white-eyes in this case) was not previously known. The family Zosteropidae includes around 100 species of small arboreal songbirds (Gill, 1971; Slikas *et al.*, 2000). These white-eyes are known for their high dispersal capabilities and have colonized more oceanic islands than any other passerine family (Moreau, 1964, cited in Gill, 1971). Although some species of white-eyes play an important role in plant pollination (Gill, 1971; Proctor *et al.* 1996; Kunitake *et al.*, 2004), white-eyes typically have a generalist diet which includes fruit, insects and nectar (Gill, 1971).

More surprisingly, the third short-spurred species of the Mascarene endemic *A.* section *Hadrangis* is regularly pollinated at night by a medium-size ortho-

pteran species from the subtribe Gryllacridinae. This recent discovery further constitutes the first recorded case of regular pollination by orthopterans in extant angiosperms (Micheneau *et al.*, in press) and Orthoptera are mostly known as a herbivorous insect order (see Micheneau *et al.*, in press, for further references on flower–orthopteran interactions).

Pollinator specificity

The observation of only a few pollinating moths per orchid species, despite the high number of potential pollinators observed in the vicinity of the orchids, revealed a high degree of specialization between long-spurred angraecoid orchids and their specific hawkmoth pollinators (e.g. Nilsson *et al.*, 1987). Similarly, it seems that each of the two bird-pollinated orchid species is specialized for efficient pollination by only one bird species (Micheneau *et al.*, 2006). This was unexpected, as bird-pollination in orchids generally involves more than one bird pollinator. For example, three hummingbird species have been reported pollinating *Sacoila lanceolata* (Aubl.) Garay (Spiranthinae Lindl. ex Meisn., Orchidoideae Eaton) in southern Brazil (*Phaethornis eurynome*, *Leucochloris albicollis* and female *Thalurania glaucopsis*) (Singer & Sazima, 2000) and, although only one hummingbird species (*Chlorostilbon maugaeus*) was proposed as the pollinator of *Comparettia falcata* Poepp. & Endl. (Oncidiinae Benth., Epidendroideae) in Puerto Rico (the only hummingbird species found in the study area), another hummingbird (*Amazilia tzacatl*) was recorded as the pollinator of this orchid in Peru (Rodríguez-Robles *et al.*, 1992). *Satyrium carneum* (Dryand.) Sims and *S. princeps* Bolus (Satyriinae, Orchidoideae) are often pollinated by two or more sunbird species in South Africa (*Nectarina* spp.) (Johnson, 1996) and, even in the unusual cases in *Disa chrysostachya* Sw. and *D. satyriopsis* Kraenzl. (Disinae Benth., Orchidoideae), in which sunbirds transfer pollen with their feet from flower to flower, at least two species of sunbirds (*Nectarina famosa* and *Cinnyris talatala*) are involved (Johnson & Brown, 2004). The lack of records of specialization in bird-pollination systems in orchids may result from the facts that (1) many birds are generalist foragers and (2) pollination by birds is difficult to observe because of their furtive behaviour (van der Pijl & Dodson, 1966). Difficulties involved in making such observations mean that the incidence of orchid–bird interactions is likely to have been underestimated.

Despite the fact that the flowers of *Angraecum cadetii* were frequently visited during both night and day by a range of flower visitors (mostly birds and nocturnal arthropods; see Micheneau *et al.*, in press, for further details), only raspy crickets were observed carrying pollinaria and pollinating orchid flowers

(Micheneau *et al.*, in press). This suggested that this unexpected interaction involved a highly specialized pollination system between the orchid and the orthopteran.

Specialization towards a pollinator group or a single specific pollinator is thought to maximize the efficiency of pollination, by reducing the risk that a generalist visitor wastes pollen in visiting foreign flowers. Especially in orchids, in which most species have their pollen packed in a unique structure, the pollinarium, that is exported in its entirety at one time by a single visitor, the loss of pollen constitutes a massive loss of the male sex function (Tremblay, 1992; Johnson, Neal & Harder, 2005; Harder & Johnson, 2008). However, if the reduction of the number of pollinators enhances the efficiency of pollination, it also results in reproduction being strongly limited by pollinators (Tremblay *et al.*, 2005), making orchids more dependent on their pollinators than the reverse (Nilsson, 1992). Such asymmetrical pollination systems are common among angiosperms (Bascompte, Jordano & Olesen, 2006) and are particularly well illustrated in the angraecoid orchids. Although long-spurred angraecoid species are often specialized to their specific hawkmoth pollinators (*P. lingens* in particular), sphingids, in turn, are generalist foragers, opportunistically exploiting a wide variety of orchids and other plants. Asymmetrical pollination systems are also evident in bird–orchid interactions. Both white-eye species are extreme generalists on Reunion, foraging on various flowers and adding fruits, seeds and insects to their diet. In addition, the flowering time of *Angraecum* spp., during which birds may survive on orchid nectar, is limited (i.e. approximately 1.5 months during the wet season).

POLLINATOR BEHAVIOUR

In all study cases, the crepuscular activity of the hawkmoths observed foraging on long-spurred flowers was extremely limited, only taking place in the evening between 17:15 and 19:30 h, whether the observations were made in Africa (Luyt & Johnson, 2001; Martins & Johnson, 2007) or in Madagascar (Nilsson *et al.*, 1985; Nilsson & Rabakonandrianina, 1988). At the same time, most observations were undertaken during these crepuscular hours (rarely after 20.00 h). Local weather conditions may affect hawkmoth foraging, which is not observed in heavy rain or strong winds (Martins & Johnson, 2007). The average duration of flower visits ranged from 1 to 7 s, lasting less than 2 s in most observations. Typically, hawkmoths, ‘smelling them in the breeze’, reached the orchids in a ‘zigzag flight upwind’, indicating a long-distance attraction by the strong scent emitted by the flowers (Nilsson *et al.*, 1987), the intensity of

which became stronger at dusk. At a short distance, hawkmoths hovered in front of the flowers, easily distinguishable by their white colour, and inserted their unrolled proboscis into the spur, pressing their heads towards the orchid column while collecting the nectar. In the majority of species, pollinaria became attached to the dorsal surface of the proboscis at a distance from the base which varied both among and within species, depending on the relationship between the length of the proboscis and orchid spur, except for *Angraecum arachnites* Schltr. in which pollinaria were attached to the ventral base of the proboscis of *Panogena lingens* because of the orientation of the orchid lip, and *Aerangis fuscata* (Rchb.f.) Schltr. in which all pollinaria were attached to the head of the moth (Table 1, Fig. 3). Martins & Johnson (2007) noted that pollinaria were often attached to the areas of the head that were worn and thus lacked scales. A moth may carry pollinaria of different orchid species and the number of pollinaria found on the proboscis of a moth was also variable (Nilsson *et al.*, 1987; Nilsson & Rabakonandrianina, 1988). As a maximum, Luyt & Johnson (2001) found 20 pollinaria of *Mystacidium venosum* within 0.6 cm of each other on a single proboscis of *Nephele accentifera*. Pollinaria attach firmly to the pollinators by their sticky viscidia, which adhere most effectively to the smooth surfaces of the proboscis instead of hairy zones of their heads (in contrast to the powdery pollen of most angiosperms). This firm fixation allows the pollinaria to remain attached to the bodies of the pollinators for long periods, which in turn allows the pollinators to disperse pollen over long distances and to ensure effective outcrossing (e.g. Nilsson *et al.*, 1985, 1987; Nilsson & Rabakonandrianina, 1988; Nilsson, Rabakonandrianina & Pettersson, 1992). For instance, Wasserthal (1997) reported that a pair of undeposited pollinia of *A. sesquipedale* remained undamaged on the base of the tongue of a female *Xanthopan morgani* var. *praedicta* for almost 25 days (see also Luyt & Johnson, 2001, for experiments on pollen longevity). In addition to the firm adhesion, bending of pollinaria has also been reported to occur in the majority of long-spurred species studied. Typically, a few minutes after the pollinarium is removed from a flower, the stipes undergo a bending movement of 90° from their initial position, changing the orientation of the pollinia, which become parallel to the axis of the proboscis, and correctly orient to allow pollen to be deposited on conspecific stigmas. These bending movements of pollinaria are assumed to preclude geitonogamy (selfing between flowers of the same plants) (e.g. Darwin, 1862; Wasserthal, 1997; Johnson & Edwards, 2000; Luyt & Johnson, 2001; Peter & Johnson, 2006).

White-eyes were active on flowers of Mascarene *Angraecum* spp. for the whole day from 06:02 h

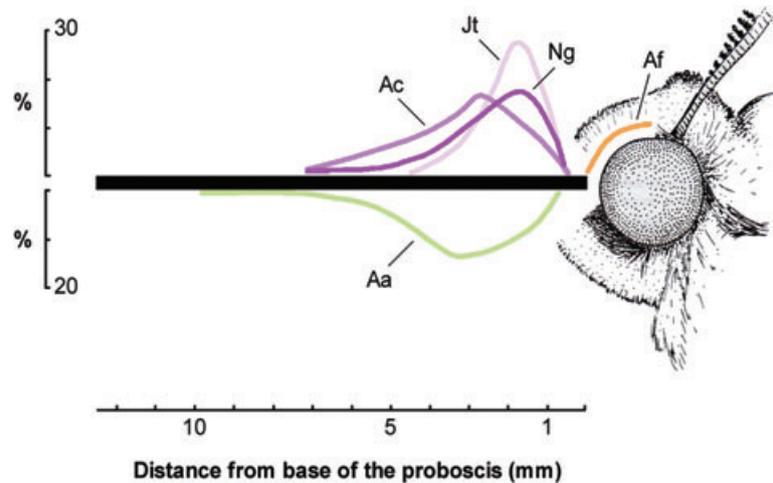


Figure 3. Attachment distributions of pollinaria of angraecoid orchids on the proboscis of *Panogena lingens*. Aa, *Angraecum arachnites*; Ac, *Angraecum compactum*; Af, *Aerangis fuscata*; Jt, *Jumellea teretifolia*; Ng, *Neobathiea grandidierana* (modified from Nilsson *et al.*, 1987).

(earliest visit recorded) to 18.05 h (latest visit recorded), whether the weather was sunny or gloomy and rainy (Micheneau *et al.*, 2006, 2008c; C. Micheneau & J. Fournel, unpubl. data). The two bird species, *Z. olivaceus* and *Z. borbonicus*, adopted the same behaviour while feeding on orchid flowers. Typically, they landed on a leaf or inflorescence and probed all 'fresh-looking' flowers within reach, moving around the inflorescence rachis if necessary. The time spent on orchids varied from 9 to 27 s (Micheneau *et al.*, 2006). To reach the nectar in the spurs, the birds had to brush against the column and to pick up pollinaria as they retreated from the flowers. Pollinarium attachment on the beaks contrasted strongly between the two orchid species in relation to their floral morphology (i.e. flower opening and spur length). Foraging on *A. striatum* involved pollinarium attachment on both the upper and lower base of the beak (Fig. 2B), whereas pollinaria were always fixed at the extreme tip of the beak for *A. bracteosum*. Although birds may carry a large number of pollinaria, it was not rare to observe them cleaning their beaks on tree fern stipes or nearby hardwood species to remove their conspicuous packages.

Raspy crickets were observed foraging on flowers of *A. cadetii* only during the darkest hours of night, between 19:50 and 04:40 h. In contrast to the furtive behaviour of both sphingids and birds, visits by these insular orthopterans could take a long time, up to 41 s for a single flower! Typically, raspy crickets reached orchid flowers by climbing up the leaves or jumping across from neighbouring plants. In all successful pollination events, the raspy cricket positioned itself on the fleshy lip of the flower with its dorsal side orientated towards the orchid column and probed

deep within the spur. Pollinaria of the orchid became stuck to the head of the crickets as they retreated from flowers' (Micheneau *et al.*, in press).

POLLINATION AND REPRODUCTIVE SUCCESS

Compilation of data from the literature indicated that pollination and fruiting success did not differ markedly between angraecoid orchids adapted to different pollinator groups, although pollination efficiency tends to be slightly higher in sphingid-pollinated flowers than in cases of bird-pollination (Fig. 4). Fruit set averaged approximately 17% for Madagascar–African moth-pollinated species, 13% for Mascarene bird-pollinated ones and 22% for Mascarene *A. cadetii* pollinated by orthopterans. Whatever the pollinator group, fruit set is however, close to the general mean of 17% reported for tropical orchids (Tremblay *et al.*, 2005). Considering each pollinator group, fruit set resulting from hawkmoth pollination ranged from 3% for *Aerangis confusa* J. Stewart (Kenya, Martins and Johnson, 2007) to 41% for *Angraecum arachnites* (Madagascar, Nilsson *et al.* 1985) and averaged 17%. This mean value is much lower than the average of c. 45% calculated from data found in the literature (Tremblay *et al.*, 2005) for tropical reward moth-pollinated orchids (for comparison, fruit set reaches approximately 51% for temperate reward moth-pollinated species; Tremblay *et al.*, 2005).

Reproductive success of bird-pollinated *Angraecum* spp. (c. 13%, Table 2) is also lower than the general median reported in the literature for bird-pollinated orchids (54%; Tremblay *et al.*, 2005). However, this level of fruit set is close to the hummingbird-pollinated *Comparettia falcata* (c. 18%), which shares

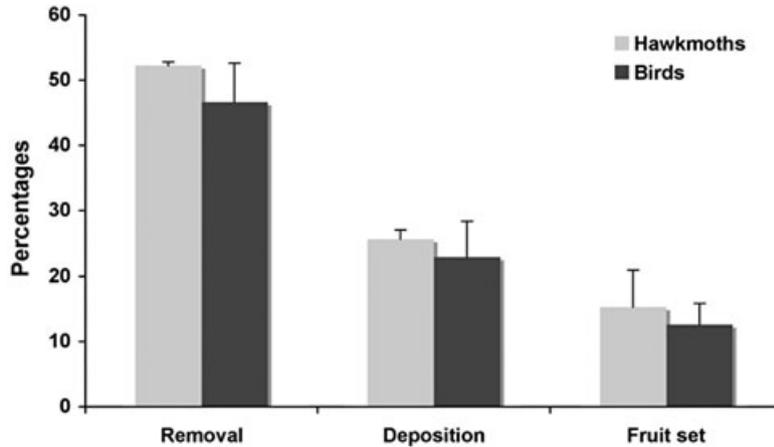


Figure 4. Comparison of pollination and reproductive success between hawkmoth- and bird-pollinated angraecoid orchids. Mean (\pm SD) of pollinaria removal, pollinaria deposition and fruit set is presented. Data were compiled from Nilsson *et al.*, 1985; Nilsson & Rabakonandrianina, 1988; Wasserthal, 1997; Luyt & Johnson, 2001; Martins & Johnson, 2007 for moth-pollinated species and from Micheneau *et al.*, 2006, 2008c for bird-pollinated species.

Table 2. Summary of floral features and reproductive success according to reproductive strategy

Pollinator groups	Hawkmoths*	Birds†	Orthopterans‡	Self-pollination§
Location	Madagascar	Reunion	Reunion/Mauritius expected	Reunion
Flower colour	White or greenish–white	White	White/cream–white	Cream–white
Flowers/inflorescence	< 5	> 5	< 5	1
Spur length (cm)	4.6–33.3‡	0.8–1.2	0.6 (mean)	13.8 (mean)
Spur shape	Filiform, or spirally twisted	Conical	Conical	Filiform
Spur opening	Narrow	Large¶	Very large	Narrow
Nectar volume (μ L)	38.9 \pm 57.1 (9)	6.3 \pm 2.0 (2)	14.5 \pm 13.7 μ L (25)	6.1 \pm 6.1 (9)
% sugar (g/100 g)	15.2 \pm 5.1 (12)	9.7 \pm 0.1 (2)	12.3 \pm 3.6 (21)	10.7 \pm 1.3 (6)
Floral scent (human nose)	Strong the evening	Not detectable	Discrete (at night only)	Not detectable
Floral scent composition	Dominated by aromatics**	No volatile compound	Dominated by (<i>E</i>)- β -ocimene at night	Indole at night
Fruit set (%)	17.0	12.6	21.7	62.8

Mean \pm SD (*N*) are presented for nectar properties. *N* corresponds to the number of species compiled from the literature, except for Orthopterans and self-pollination, for which *N* corresponds to the number of individuals sampled in the species *Angraecum cadetii* and *Jumellea stenophylla*, respectively. If variation were observed in nectar sugar concentration for a species, the highest value was chosen.

*Compiled data from Nilsson *et al.* (1985, 1987); Nilsson & Rabakonandrianina (1988); Wasserthal (1997); Luyt & Johnson (2001); Martins & Johnson (2007) (study species are *Angraecum arachnites*, *A. compactum* Schltr., *A. sororium*, *A. sesquipedale*, *Aerangis articulata* (Rchb.f.) Schltr., *A. brachycarpa* (A.Rich.) Durand & Schinz, *A. confusa*, *A. ellisii*, *A. fuscata*, *A. kotchyana*, *A. thomsonii*, *Jumellea teretifolia* Schltr., *Mystacidium venosum*, *Neobathia grandidierana* (Rchb.f.) Garay and *Rangaeris amaniensis* (Kraenzl.) Summerh).

†Compiled data from Micheneau *et al.* (2006; 2008c) (study species are *Angraecum bracteosum* and *A. striatum*).

‡Data from Micheneau *et al.* (in press) (study species is *Angraecum cadetii*).

§Data from Micheneau *et al.* (2008b) (study species is *Jumellea stenophylla*).

¶*Angraecum bracteosum* display a callus on the lip, which restricted flower’s entrance.

**Kaiser (1993).

both an epiphytic habit and a tropical distribution (Rodríguez-Robles *et al.*, 1992) with these *Angraecum* spp.

Surprisingly, flower visits by orthopterans involved a high rate of fruiting success, averaging 22% at the study site of the Plaine des Palmistes in Reunion (average for 3 years). This rate is much higher than the 10% fruit set recorded for the bird pollinated sister species at the same study site and in the same years (Micheneau, 2005; Micheneau *et al.*, 2008c).

Reproductive success seems to be generally pollinator-limited in the angraecoid orchids (e.g. Wasserthal, 1997; Luyt & Johnson, 2001; Micheneau, 2005; Micheneau *et al.*, 2006, 2008c). However, the spectacular rate of flower abortion found in *Angraecum striatum* (Micheneau *et al.*, 2006) and the low rate of fruit set in *Aerangis ellisii* (Rchb.f.) Schltr. in spite of a high frequency of both pollen removal and deposition (Nilsson & Rabakonandrianina, 1988) suggest that in some species resource allocation may also affect reproductive success.

FLOWER MORPHOLOGIES AND REPRODUCTIVE STRATEGIES

Hawkmoth-pollinated flowers

Long-spurred angraecoid orchids show a clear suite of floral adaptations to hawkmoth pollination, including floral traits involved in pollinator attraction (i.e. white flower and strong scent), pollinator fidelity (large sugar-rich nectar reward, situated in deep spurs easily accessible for long-tongued moths) and structural modifications to the column, the lip and/or the spur which all contribute to favourable contact between the head or proboscis of the moth and the orchid column and thus result in efficient pollinarium removal or deposition. The fact that in most species the lip lacks a landing platform is thought to facilitate pollination by hawkmoths, as they are able to probe nectar while hovering above the flowers without requiring any support from the flower (Luyt & Johnson, 2001). However, a landing platform may exist in some species, such as the very long-spurred *Angraecum sesquipedale* and *A. sororium*. Especially for these two species, the landing platform is thought to facilitate pollination by hawkmoths that swing-hover such as *Xanthopan morgani praedicta* and *Coelonia solani* (Wasserthal, 1997). In addition, particular shapes of lips may further function as a mechanical guide to orient the base of the proboscis into the spur (Nilsson *et al.*, 1985; Luyt & Johnson, 2001). Specific adaptations of the rostellum may also extend over the mouth of the spur, flanking the entrance of the spur, presumably forcing the head of the pollinator into close contact with the orchid column (e.g. *Aerangis elisii*, Nilsson & Rabakonandri-

anina, 1988; see also Wasserthal, 1997). Nectar production in the spur is another factor that has been viewed as being of crucial importance for successful pollination by hawkmoths (e.g. Darwin, 1862; Nilsson *et al.*, 1985). In most flowers, the nectar is located only at the base of the spur, forcing the pollinator to probe deep into the spur to reach the nectar or 'to drain the last drop' (Darwin, 1862), so that its head makes the best contact with the orchid column to ensure successful pollen export and import (e.g. Darwin, 1862; Nilsson *et al.*, 1985, 1987; Nilsson & Rabakonandrianina, 1988; Wasserthal, 1997; Martins & Johnson, 2007). In addition, spirally twisted spurs found in *Aerangis thomsonii* (Rolfe) Schltr., *A. kotschyana* (Rchb.f.) Schltr. and *Angraecum arachnites* may also serve identical purposes. Spirally twisted spurs involve tight frictions between orchid spur and hawkmoth tongue during nectar consumption, which may encourage moths to probe deeper into the spur and to stay longer in close contact with the reproductive organs of the orchid.

Bird-pollinated flowers

Although they are phylogenetically close to Malagasy species that perfectly match the sphingophilous pollination syndrome (Micheneau *et al.*, 2008a), the Mascarene bird-pollinated *Angraecum* spp. show the usual convergent adaptations observed in other bird-pollinated orchids. Except for the colour of the flower, which is pure white rather than vividly coloured as in typical bird-pollinated flowers, *A. striatum* and *A. bracteosum* display multi-flowered inflorescences of unscented and short-spurred flowers, which are rather fleshy in texture. In addition, *A. bracteosum* flowers have a strong fold (callus) on the lip, restricting the opening of the flower. This callus is a convergent feature often found in bird-pollinated orchids and may force the birds to push their beaks against the orchid column to reach the nectar in the spur (e.g. van der Pijl & Dodson, 1966; van der Cingel, 2001).

Orthopteran-pollinated flowers

Flower morphology of *A. cadetii* appears to be the result of a suite of floral adaptations for efficient pollination by raspy crickets (Micheneau *et al.*, in press). Sepals and petals are quite small and fleshy; no damage was observed on these flower parts, suggesting that orthopterans do not usually predate the orchids (Micheneau *et al.*, in press). The shape and the size of the pollinia (quite large, flat and triangular), are also atypical for the genus *Angraecum*, especially if compared with the small ball-like pollinia of the bird-pollinated species of *A.* section *Hadrangis* (Fig. 5). In addition, there is a close match in size between the head of the insect and the nectar-spur opening (Micheneau *et al.*, in press; S. Hugel, unpubl.

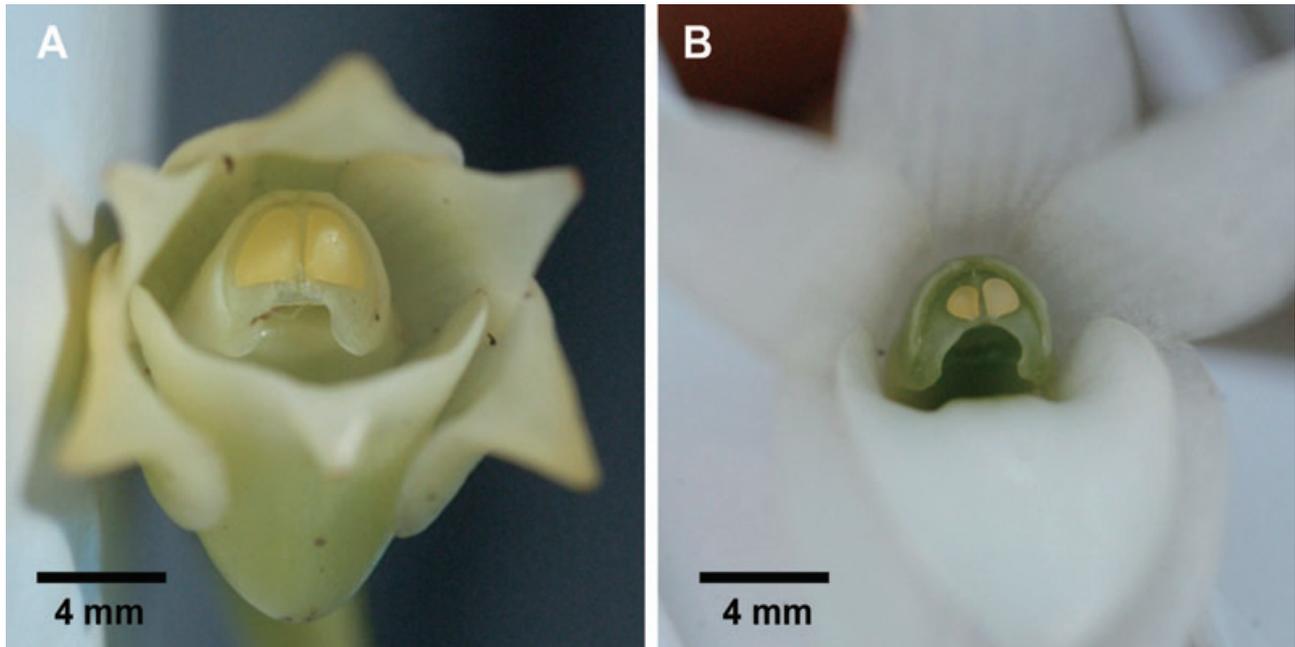


Figure 5. Comparison of the shape and the size of pollinia between bird- and orthopteran-pollinated species within *Angraecum* section *Hadrangis*. A, Orthopteran-pollinated *A. cadetii*. B, Bird-pollinated *A. bracteosum*.

data). Finally, the floral bouquet of *A. cadetii* (i.e. nocturnal emission of monoterpene-dominated scent; Micheneau *et al.*, in press) probably plays a major role in attracting raspy crickets, which use their long antennae to explore their surroundings at night (C. Micheneau, pers. observ.; S. Hugel, pers. comm.).

Self-pollinating flowers

The study of different floral traits related to plant–pollinator interactions, namely flower longevity, spur length, nectar properties and scent production, have been investigated in the autogamous long-spurred *Jumellea stenophylla* (Frapp. ex Cordem.) Schltr., which is endemic to Reunion (Micheneau *et al.*, 2008b). Results have shown that flower longevity of this self-pollinating species was much shorter (only 4–5 days) than that of hawkmoth-pollinated orchids (c. 25 days, Luyt & Johnson, 2001). This short flower lifetime was associated with autonomous self-pollinations that activated flower senescence. Although spur length of *J. stenophylla* (c. 13.8 cm) is within the reported spur size of long-spurred angraecoid orchids studied, the species only produces a relatively small amount of nectar (c. 6.1 μ L) and flowers do not emit a strong and sweet scent at dusk (see Micheneau *et al.*, 2008b for further details). This lack of floral scent, associated with the high degree of auto-pollinated flowers with a short lifespan, makes the flowers highly unattractive for pollinators and

consequently precludes any chance of this orchid being cross-pollinated.

EVOLUTION OF POLLINATOR-LINKED FLORAL TRAITS

Nectar properties

Nectar volume and sugar concentration in angraecoid orchids show clear patterns in relation to pollinator group. In hawkmoth-pollinated species, nectar sugar concentration averages 15.2%, whereas it is only 9.7% in bird-pollinated flowers (Table 2). The values for hawkmoth-pollinated angraecoid orchids are similar to those found in hawkmoth-pollinated plants more generally (Pyke & Waser 1981) and the dilute nectar found in angraecoid orchids conforms almost exactly to the pattern observed for other plants pollinated by short-billed opportunistic birds (Johnson & Nicolson, 2008). Plants pollinated by specialist flower feeding birds (hummingbirds and sunbirds) tend to produce nectar with a sugar concentration around 20%, whereas those pollinated by short-billed opportunistic birds tend to produce nectar with much lower concentrations around 10% (Johnson & Nicolson, 2008).

Gradients in nectar sugar concentrations along the length of the nectar column were recently reported to occur within spurs in African *Aerangis* and *Rangaeris* spp. (Martins & Johnson, 2007). In all study species, nectar was significantly more diluted towards the mouth of the spur than at the tip (concentrations ranged from c. 1% at the proximal end of the spur to

c. 20% at the distal end). Nectar sugar gradients may have the same function as spirally twisted spurs and the presence of the nectar only in the lower part of the lip, i.e. they encourage the moth to go as deep as possible into the spur while probing nectar, enhancing at the same time the chance to ensure efficient pollination (Martins & Johnson, 2007). Post-pollination reabsorption of sugars from spurs has also been reported for two aerangoid orchid species (Koopowitz & Marchant, 1998; Luyt & Johnson, 2002) and this may mobilize resources for fruit development (Koopowitz & Marchant, 1998; Luyt & Johnson, 2002).

The sugar composition of nectar of only one angraecoid species has been investigated so far (*Mystacidium venosum*, Luyt & Johnson, 2001). The nectar of this hawkmoth-pollinated orchid proved to be especially sucrose-rich (87% sucrose, 10% fructose and 3% glucose). It would be worthwhile in future research to develop further these comparative studies of nectar sugar composition, with the aim of investigating evolutionary patterns of this floral trait between bird- and moth-pollinated species within the angraecoid orchid group (Johnson & Nicolson, 2008). In particular, one could then test the prediction that there should be an association between opportunistic bird pollinators and hexose-dominated nectar, based on the inability of these birds to digest simple sugars (e.g. Martínez del Río, Baker & Baker, 1992).

Long nectar spur evolution

Darwin's seminal work on the angraecoid orchid *Angraecum sesquipedale* triggered the beginning of a long debate about the evolution of long floral spurs. The two competing explanations for these traits are the Darwinian 'coevolutionary race' model (1862) and the 'pollinator shift' model developed by Stebbins (1970). The Darwinian 'coevolutionary race' model attributes adaptive elongation of the tongues of the moths and the spurs of the flowers to reciprocal coevolution, as a result of a gradual increase as a result of mutual benefit (gain in reproduction efficiency for the orchid and gain in nectar foraging efficiency for the hawkmoth). The alternative 'pollinator shift' model states that long tongues in hawkmoths were present before the long spurs of the orchids and evolved without any reciprocal interactions with them (Wasserthal, 1997; Whittall & Hodges, 2007). This model attributes the evolution of long nectar spurs to punctuated shifts to successive pollinators with longer tongues. Both models agree, however, with Darwin's idea that longer spurs evolved under directionally selective pressure exerted by long-tongued pollinators. Darwin did not exclude the possibility that longer tongues in hawkmoth may have evolved from selection on body size 'in relation to

their conditions of life, either in the larval or mature state' (Darwin, 1862) or in response to the spur length of other plants. Much more controversial has been Wasserthal's (1997) assertion that the evolution of long tongues in hawkmoths was driven by selection imposed by flower predators, rather than availability of nectar in deep tubular flowers (e.g. Wasserthal, 1997; Nilsson, 1998a, b; Svensson, Rydell & Töve, 1998; Wasserthal, 1998).

Because the interactions between angraecoid orchids and hawkmoths are mostly asymmetric (Nilsson & Rabakonandrianina, 1988), it seems likely that coevolution was diffuse at the guild level and not strictly pairwise. It also seems likely that many members of these guilds became adapted to long-tongued hawkmoths via a pollinator shift rather than through coevolution. However, the relationships between long-spurred angraecoid orchids and their hawkmoth pollinators remain largely unknown if we consider the small number of species that has been studied (< 20 species) against the number of existing species (> 760 species in Angraecinae *s.l.*, i.e. including Aerangidinae). We need to expand our knowledge to better assess the evolutionary outcomes between these orchids and their pollinators. In particular, fine-scale studies of geographical variation in mutualistic traits such as tongue length and nectar depth may reveal co-evolutionary processes at the population level (see Anderson & Johnson, 2008). However, much of the evolutionary potential of these orchids and their pollinators may already have been lost as a result of the massive destruction of primary forests in Madagascar and the large number of fragmented populations (i.e. reduction in population size and genetic diversity can have a severe effect on the evolutionary potential, e.g. Hubbell *et al.*, 2008).

CONCLUDING REMARKS

Orchids should be studied more because they epitomize evolution in its most dynamic aspect, the rapid production of an incredibly diverse array of species (Chase *et al.*, 2003).

Orchids have been a model system for studying floral adaptation and diversification ever since Darwin's influential book on the subject was published in 1862. They provide some of the most exceptional examples of highly specialized interactions between animals and plants. Not surprisingly, orchid pollination has been intensively studied relative to that in other extant angiosperms, with entire books dedicated to the subject (e.g. Darwin, 1862, 1877; van der Pijl & Dodson, 1966, van der Cingel, 1995, 2001), book chapters (e.g. Proctor *et al.* 1996, Roberts, 2003; see also Dressler, 1981, 1993) and numerous high-impact publications (e.g. Nilsson, 1983b, 1992; Romero & Nelson,

1986; Nilsson *et al.*, 1992; Johnson & Nilsson, 1999; Schiestl *et al.*, 1999, 2003; Cozzolino & Widmer, 2005; Liu *et al.*, 2006; Ramírez *et al.*, 2007).

The unique, unexpected and diverse orchid pollination systems provide excellent opportunities for determining how pollinator-mediated selection has shaped the evolution of floral traits (e.g. flower scent, colour and spur length) and how shifts between pollinators may be responsible for patterns of macroevolution at the species level and above. However, in spite of the large size of this model family and the long history of orchid pollination biology, the identity and specificity of most orchid pollinators are unknown and remain inadequately studied, especially in the tropics where the family has undergone extensive diversification.

Finally, highly specialized pollination interactions, such as those exemplified by orchids, are highly vulnerable to changes in populations and habitats. As Darwin pointed out while the pollinating sphingid of *A. sesquipedale* was still undiscovered: 'If such great moths were to become extinct in Madagascar, assuredly the *Angræcum* would become extinct' (Darwin, 1862). Once again, Darwin led the way in drawing this important connection between evolutionary studies and the 'real-world' problem of conservation. This is especially relevant today because of the present context of pollinator decline (Buchmann & Nabham, 1996; Kearns, Inouye & Waser, 1998; Biesmeijer *et al.*, 2006; Ghazoul, 2006) as a result of habitat loss (Harris & Johnson, 2004; Aguilar *et al.*, 2006) and climate change (Deutsch *et al.*, 2008).

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