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## Pollination biology of the endemic orchid *Vanilla bosseri* in Madagascar



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## Abstract

The pantropical orchid genus *Vanilla* has a diverse floral morphology and there is a lack of knowledge about the pollination biology of its species in all geographical areas except the American tropics. The present study investigated the pollination biology of the endemic *Vanilla bosseri* Allorge in a dry, deciduous forest near Morondava at the west coast in Madagascar. The total and flower producing population was estimated. Floral measurements and visitor observations were conducted and the floral phenology and anthesis were investigated. A breeding experiment with three treatments, cross-pollination, self-pollination and pollinator exclusion, suggest that *V. bosseri* is allogamous but self-compatible, and dependent on pollinators for its reproduction. Natural fruit set was low, 3.96%, less than one-tenth than that of hand-pollinated flowers, suggesting the species is pollen limited possibly due to low pollinator visitation. Flower morphology clearly indicates that the species is bee pollinated. The study shows that the orchid acts by deceit and attracts pollinators through generalized food flower deception. The main visitor, the allodopine bee *Macrogalea ellioti*, is most likely not the effective pollinator, but instead a pollen thief that occasionally acts as a pollinator. Floral morphology and pollination mechanism indicate that an effective pollinator would have an operative size similar to that of medium sized bees such as *Lithurgus pullatus* or *Tetraloniella madecassa*, but further studies are needed to reveal the relative impact of accidental and regular pollinator bee species.

**Keywords:** *Vanilla* – endemic orchid– pollination – pollen thief – breeding system – Madagascar

## Introduction

### The *Vanilla* genus

The orchid genus *Vanilla* Miller is pantropical and found on all continents except for Australia. It comprises some 107 species in the genus, which represents half of all species in the subfamily Vanilloideae. There are 54 species in tropical America, making this the most diverse location, followed by 28 species in southeastern Asia and New Guinea and 25 species in Africa-Madagascar (Soto Arenas and Cameron 2003, Allorge 2013).

### Life form

Most *Vanilla* species are vines but some occur as shrubs (Dressler 1981). Some species are epiphytes and complete their entire life cycle in tree crowns but many are hemiepiphytes that begin their life on the ground and then climb trees, however, they usually do not reach the upper canopy. Many of the hemiepiphytes can also be found climbing on rocks (Soto Arenas and Cameron 2003). Some species are saprophytic (Dressler 1981). The roots, formed at each stem node, are generally flattened, slender and smooth when they are above ground but when in soil they are thick, terete and villose (Soto Arenas and Cameron 2003). The stems usually have a climbing habit and are woody or fleshy. The leaves are distichous or spirally arranged and depending on the species they can vary between membranaceous, coriaceous or fleshy (Dressler 1981). Some species develop conspicuous and persisting leaves while others appear leafless but have scale-like deciduous leaves (Soto Arenas and Cameron 2003). The flower is generally short lived and when it has been fertilized the perianth is usually



shed. In some *Vanilla* species with membranaceous leaves the flower remains open longer, for two to three days (Soto Arenas and Cameron 2003).

#### *Habitat*

The habitats of the *Vanilla* species are very different, ranging from subtropical to equatorial forest (Soto Arenas and Cameron 2003). Those species that have been recorded at higher altitudes have primarily been found in dry-forest intermountain valleys, savanna-like vegetation or montane rain forest. Leafless *Vanilla* species grow primarily in dry areas whereas species with leaves usually grow in more humid places. Especially species with membranaceous leaves are found in moister environments. Their brittle and not fleshy stems combined with a high stomata density on the leaves seem to be traits that are not compatible with establishment in seasonal areas. Despite the habitat differences all *Vanilla* species are thermophilous (Soto Arenas and Cameron 2003).

#### *Floral morphology*

The inflorescences of *Vanilla* species are axillary, racemose to paniculate or rarely a complex cyme. It can either be elongated, bearing leaf-like bracts and remote flowers or congested with scale-like bracts, or an intermediate variant of these two extremes (Soto Arenas and Cameron 2003). *Vanilla* species have a diverse floral morphology but most flowers are showy, gullet-shaped and produced in succession. The flowers are white or yellow (Cribb and Hermans 2009), and some are strongly fragrant (Soto Arenas and Cameron 2003). The flowers are resupinate, as most orchid flowers are, and there is usually an abscission layer between the ovary and the perianth (Dressler 1981). The petals are typically dorsally keeled and both sepals and petals are free and spreading (Soto Arenas and Cameron 2003). The lip is often partially united with the column margins (Dressler 1993), which creates an open pouch or funnel-shaped chamber (Soto Arenas and Cameron 2003). Many species have a penicillate callus on the lip that is formed by a cluster of retrorse scales (Soto Arenas and Cameron 2003). The column is slender with a terminal anther that is often subtended by a distinct filament (Soto Arenas and Cameron 2003). The stigma is variable, but usually entire or three-lobed, and generally emergent (Dressler 1981). In some species the midlobe of the stigma act as a rostellum and produces a narrow band of sticky material along the margins, which creates a diffuse viscidium. The rostellum is inflexed and hides the lateral receptive stigma lobes (Rasmussen 1985). The pollen of *Vanilla* is soft, mealy, sticky and not at all divided and is shed as monads (Dressler 1981). It does not form distinct pollinia and has been observed to be transferred as a flat, triangular pad on the scutellum of *Eulaema* and *Euglossa* bees (Fig. 1) (Dressler 1993). The fruit is often fleshy, one- or three-locular and forms two unequal valves when opened (Dressler 1981). In some species it is strongly fragrant (Soto Arenas and Cameron 2003). The seeds are relatively large within the family (Soto Arenas and Cameron 2003), have a hard seed coat and some have prominent wings (Dressler 1981).

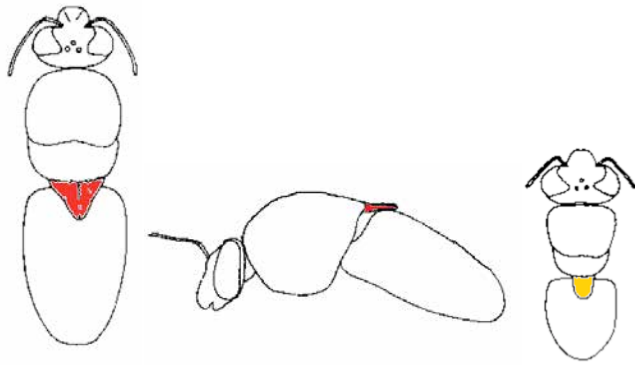


Figure 1. Example of the placement of the pollen on the pollinator. To the left an *Euglossa* species with pollen from *Vanilla fragrans* Ames (pollen marked red) and to the right an *Eulaema* bee with pollen from *Vanilla pompona* Schiede (pollen marked yellow). Adapted from Ackerman (1983).

### *Pollination system*

Because of unusually high fruit sets a few *Vanilla* species have been suggested to be self-pollinated through stigmatic leak or a reduced rostellum (Soto Arenas and Cameron 2003, Householder *et al.* 2010, Soto Arenas and Dressler 2010, Van Dam *et al.* 2010), but the majority are dependent on pollinators (Soto Arenas and Cameron 2003). The most common reward for orchid pollinators is nectar (Dressler 1993), but no *Vanilla* species are known to produce floral nectar (Gigant *et al.* 2011). There are many other possible rewards for orchid pollinators such as floral fragrance and pseudopollen (Dressler 1993), oil and rarely pollen or stigmatic exudates (van der Pijl and Dodson 1966). Several *Vanilla* species in the American tropics are pollinated by euglossine bees (Dressler 1981), a tribe that is known to collect flower fragrance from non-nectar producing orchids (Dodson *et al.* 1969). So far there is no direct evidence for this scent collecting behaviour in any *Vanilla* species (Gigant *et al.* 2011). Many orchids have also developed different types of deceptive systems to attract pollinators (van der Pijl and Dodson 1966), a strategy known to be used by several *Vanilla* species in the American tropics (Soto Arenas 1999, Soto Arenas and Cameron 2003).

The general floral structure of *Vanilla* indicates that its species are primarily bee pollinated (Dressler 1981). The euglossine bees that pollinate many *Vanilla* species in the American tropics do not occur in the Old World tropics. Most likely the pollinators of native *Vanilla* in the Old World are other large bees (Dressler 1981). Presently there is a considerable lack of knowledge about the actual pollinators in all geographical areas except the American tropics. There is, still, a strong morphological convergence between some American and Asian *Vanilla* species, which implies that the Asian bees that pollinate may be largely similar in several ways to the American euglossine bees (Soto Arenas and Cameron 2003).

### **The Malagasy *Vanilla* species**

There are seven endemic *Vanilla* species in Madagascar, not including the widely cultivated *Vanilla planifolia* Andrews that is native to Mexico (Du Puy *et al.* 1999, Allorge 2013). All grow as scrambling vines along tree trunks and in some case rocks. Two species, *V. coursii* H. Perrier and *V. francoisii* H. Perrier, occur solely in humid forests and are the only Malagasy species with leaves (Perrier de la Bâthie 1939). *Vanilla coursii* occurs in the southwest of the island on sandy soils in humid, low altitude forest and *V. francoisii* grows in costal forest in the north-eastern parts (Cribb

and Hermans 2009). The leaves in the five other species are reduced to scales and they therefore appear leafless. They belong to the Aphyllae section of the genus (Rolfe 1896). This section has recently been shown to be a paraphyletic group and leaflessness has evolved at least three times within the genus as an adaptation to dry conditions (Bouetard *et al.* 2010). *V. humblotii* Rchb. f. and *V. perrieri* Schltr. both have yellow flowers. The former occurs and grows on rocks and low-elevation vegetation in the north of Madagascar and the Comoro Islands while *V. perrieri* occurs and grows in seasonally dry, deciduous forest with sandy soils in parts of western and south Madagascar (Perrier de la Bâthie 1939). *V. madagascariensis* Rolfe, *V. decaryana* H. Perrier and *V. bosseri* Allorge all have white flowers and are mainly distinguished by the difference in flower size (Allorge 2013). *V. madagascariensis* is the largest and most widespread of the species. It can be found all along the west coast in coastal forest, humid evergreen forest and also in dry forest in the east. *V. decaryana* has the smallest flowers and occurs and grows in dry, deciduous scrubs among *Didieraceae*, a family endemic to south and southeast Madagascar (Perrier de la Bâthie 1939). Almost nothing is known about the pollination of the Malagasy *Vanilla* species (Allorge 2013). Preliminary observations in the field suggest that *V. madagascariensis* is pollinated by small or medium-sized bees (E. Rabakonandrianina pers. comm. to L.A. Nilsson). In Mayotte (Comoro Islands) the most common visitor to *V. humblotii* is an allodapine bee, *Allodape obscuripennis* Strand, but it has not been confirmed to act as a pollinator (unpublished data, in Gigant *et al.* 2014).

### **Study goal**

Very little is known about the biology of *Vanilla* species in Madagascar and so far nothing about their pollination (Allorge 2013). The goal of the present study was to investigate the pollination mechanism and pollinators of the endemic Malagasy species *Vanilla bosseri*. Especially the following questions were addressed: (1) What is the breeding system, (2) What principal adaptations for pollination are at work, and (3) Which is the effective pollinator or group of pollinators?

## **Method**

### **Study site and time**

Kirindy forest comprises a 12.500 ha deciduous, seasonally dry forest concession located some 20 km from the west coast of Madagascar, about 60 km northeast of Morondava (Fig. 2). It is managed by the CNFEREF (Centre National de Formation, d'Etude et de Recherche en Environnement et Forestière) but the area lacks any official protection, even though it is one of the largest remaining areas of dry deciduous forest in Madagascar (Kappeler and Fichtel 2012). The area is characterized by a long dry season followed by a hot wet season between November and March (Fig. 3). This gives the area a semi-arid character with an extremely xerophytic vegetation that is rich in endemic genera and species. The vegetation is characterized by drought adaptations such as reduced leaf size or leaflessness, thickened stems and deciduousness (Rauh 1973). The forest has a dense continuous canopy layer that is about ten meters high and a discontinuous layer of trees up to 20 meters or more. Visually the forest is spectacularly dominated by three species of baobab *Adansonia* spp. (Malvaceae). The continuous canopy layer includes species such as *Commiphora* (Burseraceae), *Terminalia* (Combretaceae), *Delonix*, *Tamarindus* (Fabaceae), *Givotia* (Euphorbiaceae), *Gyrocarpus* (Hernandiaceae) and

*Pachypodium* (Apocynaceae). The undergrowth is very dense and includes *Grewia*, *Byttneria*, *Hildegardia* (Malvaceae), *Euphorbia*, *Croton* (Euphorbiaceae), *Acacia*, *Chadsia* (Fabaceae), *Salvadora* (Salvadoraceae), *Pandanus* (Pandanaceae), *Uncarina* (Pedaliaceae) and *Henonia* (Amaranthaceae) among other species. Common vines include Apocynaceae, *Xerosicyos perrieri* Humbert (Cucurbitaceae), *Cyphostemma* spp. (Vitaceae) and *Vanilla* spp. (Koechlin *et al.* 1974).

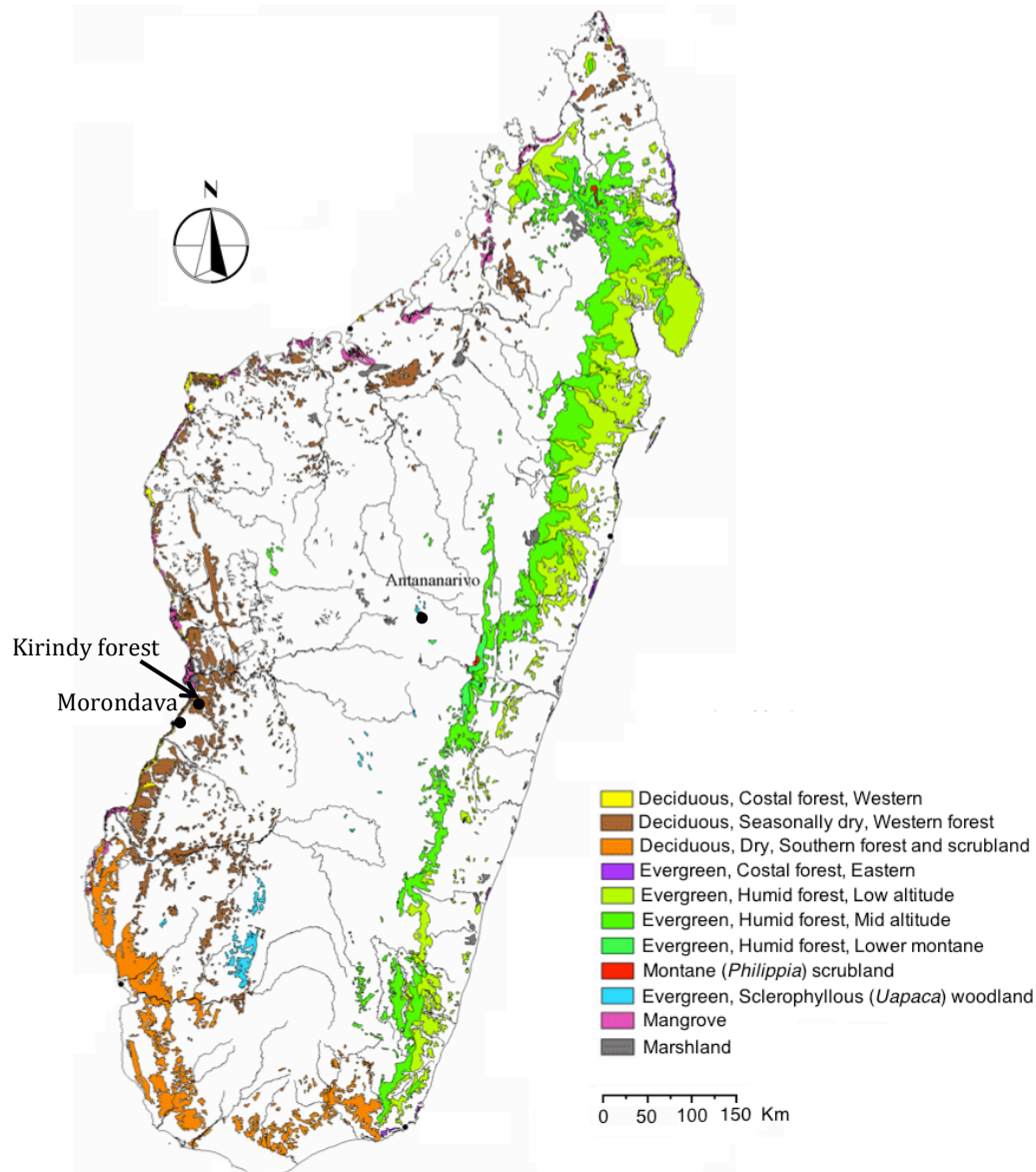


Figure 2. The remaining primary vegetation of Madagascar based on satellite images from the 1970's when 18% of the primary vegetation remained. In the late 1990's only 9.9% remained, mainly in the eastern parts of the island (Myers *et al.* 2000). The location of Kirindy forest is indicated. Adapted from Du Puy and Moat (1996).

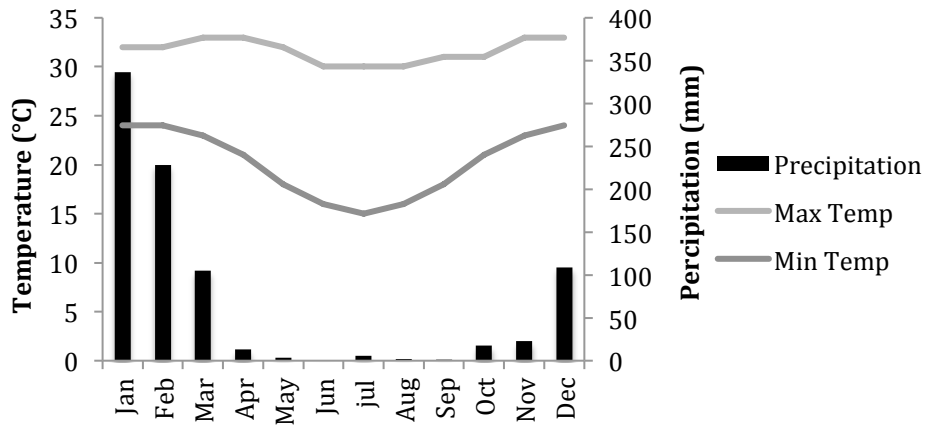


Figure 3. The climate at Kirindy forest. Mean monthly precipitation (mm), maximum and minimum temperature (°C) are shown (Worldweatheronline 2015).

The study was conducted at the end of the dry season between 13 October and 15 November 2014 in the forest surrounding Kirindy camp (Fig. 4). The study area was approximately 60 ha. Throughout the entire study the environment was very dry, with light rains on two separate occasions. The temperature varied considerably from about 15°C at night up to 35°C during the day. Few species were in bloom during the study period. In addition to *Vanilla bosseri* these included *Hildegardia erythrosiphon* Kosterm. (Malvaceae), *Combretum* sp. (Combretaceae), *Uncarina* sp. (Pedaliaceae), *Amorphophallus* sp. (Araceae), *Cordyla madagascariensis* R. Vig., *Albizia* sp. (Fabaceae) and the introduced *Azadirachta indica* A. Juss. (Meliaceae).



Figure 4. Dry, deciduous forest surrounding Kirindy camp. Note the extreme density.

### Study species

The species *Vanilla bosseri* was described recently by Allorge (2013). It is a vine with leaves reduced to scales and belongs to the Aphyllae section. The plant scrambles from the ground along shrubbery, tree trunks and canopy up to a height of ca 8 m (Fig. 5). The stem is grey-green, mostly smooth and with four grooves, two of which are more distinct, along each section. The stem sections are about 12 cm long and 1-2 cm wide. It has a clear sap. Most inflorescences occur at a height of 3-5 meters and an inflorescence produces about a dozen white, fragrant flowers. Both petals and sepals



are white, free and spreading. The white labellum is partially fused with the column and forms a funnel that becomes gradually yellow on the inside (Fig. 5). It has two pink crests that turn progressively whiter towards the base. They are ca 1 cm long and the furrow between them has short dark orange hair-like growths (Fig. 8E, H). Apart from the flower size the lack of long pubescence on the labellum is one of the most distinguishing characters between *V. bosseri* and the two other white-flowered Malagasy *Vanilla* species (Allorge 2013). Its habitat is dry, deciduous forests and according to Allorge (2013) *V. bosseri* is known from only two areas, the Namoroka reserve southwest of Mahajanga and the forests northwest of Morondava. But there is reason to believe that this species is more widespread since it is very similar to the other two white flowered species, *V. madagascariensis* and *V. decaryana*, and can easily be confused with them (Allorge 2013).



Figure 5. Inflorescences of *Vanilla bosseri* in its natural habitat in Kirindy forest. **A:** Author in the process of conducting pollination experiments. **B:** Vines scrambling along a tree trunk with three active inflorescences. **C:** Active inflorescence with the flower facing the sky in a characteristic way. **D:** Active inflorescence with ovaries from three aborted flowers still attached and buds of different sizes.

### *Total and flower producing population*

A path grid-net existed in the forest surrounding Kirindy camp. This was used to select 1 ha (Fig. 7) by using a random number generator to estimate the total population in the study area. Nine line-transects were placed with ten meters apart across the 1 ha area and all *V. bosseri* individuals along these transects were recorded on 1 November. This was then extrapolated for the total 60 ha area included in the study. One individual was defined as one or more roots climbing up along the base of a tree trunk from the ground (Fig. 6). It was difficult to distinguish individuals and this definition reduced the risk of counting the same individual multiple times, but instead there is a potential that the number of individuals were underestimated.



Figure 6. Roots of *Vanilla bosseri* climbing the base of a tree trunk, indicated by arrow to the right, then turning into vines. The two examples illustrate the definition of an individual as used in the present study.

To estimate the flower producing population, 26 patches that were accessible by walking were marked within the 60 ha study area (Fig. 7) and all *V. bosseri* individuals on each patch were counted using the same definition for one individual as above. All patches were then visited on four occasions in late October and the beginning of November with nine days apart. At every census walk the number of flower producing individuals were counted as well as the number of individuals producing fruit. It was not possible to distinguish between inflorescences from previous years and this year's inflorescences without fruits. Therefore only individuals producing either flowers or fruits during the time of the study were included in the flower producing population.



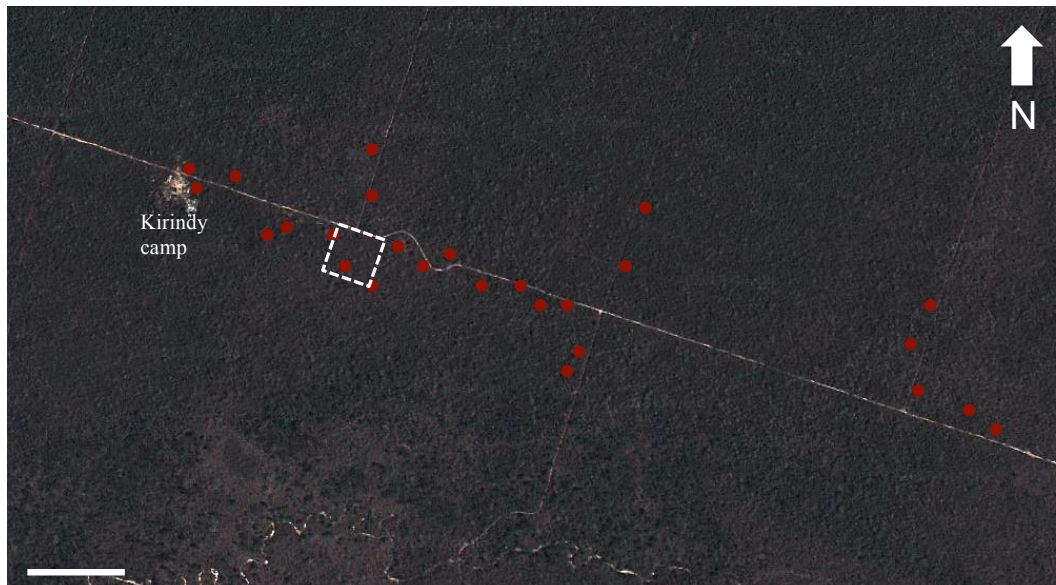


Figure 7. Kirindy camp with surrounding forest. The randomly selected 1 ha used to estimate the total population is marked with a dotted white line and the 26 patches used in the study are marked with red dots. Scale: 200 m. Satellite image from Google maps.

## Reproductive biology

### *Floral morphology*

Nine flowers, one randomly selected flower per inflorescence, from seven individuals in different patches were collected for floral measurements. The floral characters were measured within one hour after collection using a digital caliper to the nearest 0.1 mm (Fig. 8). The average number of inflorescences per individual ( $N=19$ ) and number of flowers per inflorescence ( $N=32$ ) were also counted. Four flowers were pressed and have been deposited as voucher specimens at the Museum of Evolution (UUZM), Uppsala University, Sweden.

### *Phenology and anthesis*

Thirteen of the 26 patches were visited daily from 15 October to 14 November, a total of 29 days, to investigate the phenology. The number of inflorescences per individual was counted and each bud was given an identification number. For each visit the changes were noted using the stages “bud”, “flower”, “wilted flower”, “aborted flower” and “developed fruit”. “Wilted flower” was defined as a closed flower that had not yet fallen off or developed into a fruit, “aborted flower” when the flower and ovary had shrivelled and fallen off, and “developed fruit” when the ovary showed distinct swelling. The anthesis was investigated on six different days by measuring the flower diameter (Fig. 5) of the opening and closing flower ( $N=10$ ). This was done once every half-hour from 7 a.m. until the flower closed in the late afternoon. The ambient temperature, weather and fragrance of the flower were also recorded at each census.

### *Natural fruit set*

By averaging the total number of fruits per the total number of flowers produced the mean natural fruit set was estimated. All flower producing individuals found on the 26 patches was used, altogether 18 individuals with a total of 31 inflorescences. Floral scars after any fallen ovary on the inflorescences were included to estimate the total number of flowers.



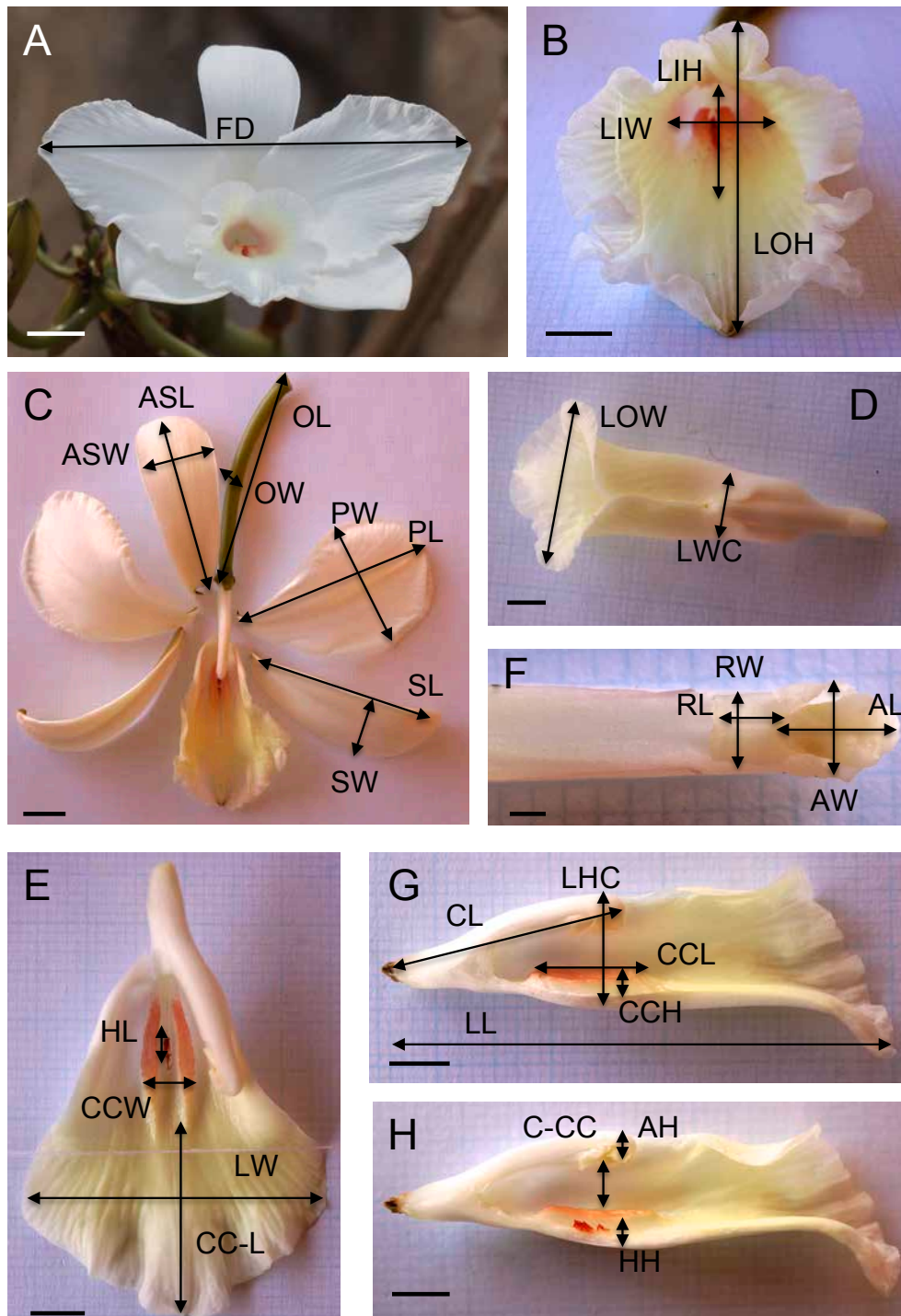


Figure 8. Floral morphology of *Vanilla bosseri*. Measured characters are shown. **A:** Flower, front view. Flower diameter (FD). Scale: 10 mm. **B:** Labellum, front view. Inner width (LIW), height (LIH) and outer height (LOH). Scale: 5 mm. **C:** Adaxial sepal length (ASL) and width (ASW), ovary length (OL) and width (OW), petal length (PL) and width (PW), lateral sepal length (SL) and width (SW). Scale: 10 mm. **D:** Labellum, dorsal view. Outer width (LOW) and width at column apex (LWC). Scale: 5 mm. **E:** Open labellum dorsal view. Length of hair-like growths on labellum (HL), central crest width (CCW), labellum width when cut open (LW), distance between central crest and labellum tip (CC-L). Scale: 10 mm. **F:** Intact column in strict ventral view. Anther length (AL) and width (AW), rostellum length (RL) and width (RW). Lateral stigma lobes concealed by rostellum. Scale: 1 mm. **G:** Longitudinal section of labellum, both central crests present. Labellum length (LL) and height at column apex (LHC), column length (CL), central crest length (CCL) and height (CCH). Scale: 5 mm. **H:** Longitudinal section of labellum, left central crest removed revealing dark orange tuft-like outgrowths. Distance between column apex and central crest (C-CC), hair-like growth height (HH) and anther height (AH). Scale: 5 mm.

### *Flower visitor observations*

The flowers of *V. bosseri* had a lifespan of one day, opening shortly after sunrise and withering during the late afternoon. Observation of flower visitors was conducted for 16 days during the end of the flowering season of the population between the 14 October and 13 November 2014. The behaviour of the visitors was observed in as much detail as possible, including their approach, entering and leaving the flower, and the time spent in the flower. Fifty-four flowers were observed for a total of 92.5 hours. Binoculars were used for most observations. Insect visitors were sampled for identification and morphological measurements of their operative size. Capture was attempted when the visitor left the flower, but proved difficult because of the height where the flowers were situated. Captured insects have been deposited as voucher specimens at Museum of Evolution (UUZM), Uppsala University. A camera (Canon EOS7D with TAMRON 70-300 mm) mounted on a tripod was used to record visitors for two days in the end of the observation period. Recordings were conducted for five minutes every half-hour between 7:30 and 11:30 am.

### *Measurements of flower visitors*

Morphological measurements of the operative size of the bees caught on *V. bosseri* were conducted to the nearest 0.5 mm. The length and height of the thorax and the total length of the bee were measured. The functional height, which is the approximate height at which the insect walks past the column, was estimated based on the leg length and overall size of the insect (Fig. 9). To make a more complete, although approximate, comparison possible with the local bee fauna, measurements of nine bee species caught in Kirindy forest by L.A. Nilsson in late November 1989 were also conducted. One specimen of typical size in each species was measured.



Figure 9. Allodapine bee *Macrogalea ellioti* with morphological measurements of thorax length, thorax height and total body length indicated with arrows. The functional height approximated by a dotted arrow. Scale: 5 mm.

### *Breeding system*

To evaluate the breeding system in *V. bosseri*, a pollination experiment with three treatments was conducted in late October and early November. (1) To assess self-fertility all insect visitors were excluded by covering seven buds just about to open with a 1x1 mm mesh net. (2) Hand self-pollination was conducted on seven flowers that had been caged with fine mesh net when still buds and re-caged after treatment. (3) Cross-pollination was performed on seven flowers by extracting pollen with a toothpick from an open flower and then placing it on the stigma of a flower of a different individual. Because of shortage of open flowers the pollen was stored in plastic zip lock bags for a maximum of 24 hours. To ensure cross-pollination between individuals the pollen used was taken from a different patch than the cross-pollinated flower. A total of ten individuals were included in the experiment and at the most three treatments per inflorescence were used to avoid possible limitation of fruit set due to resource allocation. The result was recorded 5-14 days after performed treatment. A successful pollination in *Vanilla* results in the ovary starting to swell up within a few days and unsuccessful pollination results in the flower falling off and leaving a scar (Soto Arenas and Dressler 2010). A Fischer exact test was used to conduct comparisons between the self- and cross-pollination treatments, the hand-pollination treatments and the natural fruit set.

## Results

### **Total and flower producing population**

Twenty-six individuals were found in the randomly selected 1 ha. An extrapolation thus suggested that there were approximately 1560 individuals within the 60 ha study area. The great majority, 95.2%, of the individuals were in a vegetative state, i.e. showed no sign of having produced an inflorescence during the time of the study. The number of bud, flower and fruit producing individuals in the 26 patches (Fig. 7) are given in Table 1. During the study period the number of flowering individuals decreased suggesting that the study was conducted past the maximum of the flowering season. Also the fruit set, i.e. the percent flowers that were naturally pollinated, decreased after 27 October during the study period (Fig. 10).

Table 1. The number of bud, flower and fruit producing individuals of *Vanilla bosseri* at four dates in the 26 patches. There were a total of 248 individuals, out of which 18 individuals produced active inflorescences and at the most 13 individuals at one given date.

<b>Date</b>	<b>Vegetative individuals</b>	<b>Bud and flower producing individuals</b>	<b>Fruit producing individuals</b>
2014-10-18	235	13	5
2014-10-27	236	11	8
2014-11-05	236	8	9
2014-11-14	237	5	8

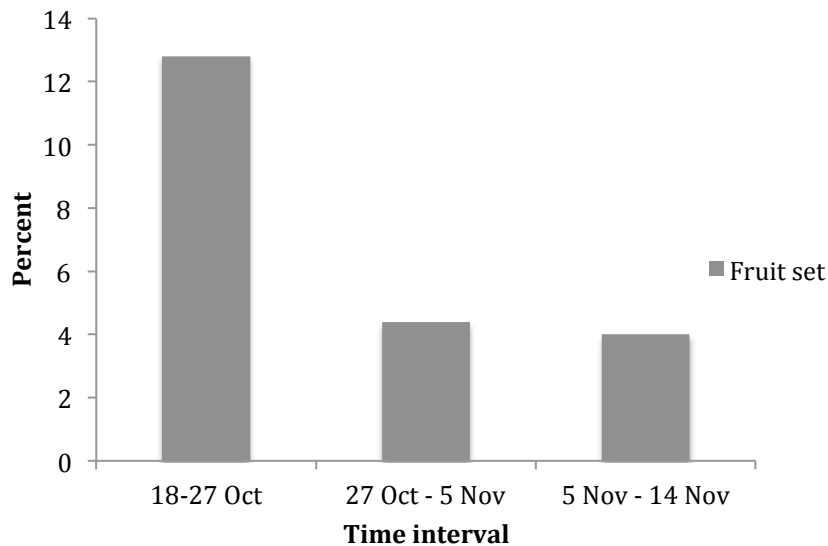


Figure 10. The fruit set for three time intervals. Based on a total of 18 reproductive individuals of *Vanilla bosseri*.

As the number of flowering individuals decreased so did the number of active inflorescences, i.e. inflorescences that produced either or both flowers and fruits. In conjunction with this the number of buds and open flowers decreased as the amount of aborted flowers increased (Fig. 11). When the study started there were already 18 maturing fruits present and another nine started to develop during the time of the study. However, ten fruits aborted, two as a result of an external damage from herbivory by insects and eight without any visible reason. Accordingly, the total number of fruits decreased during the time of the study.

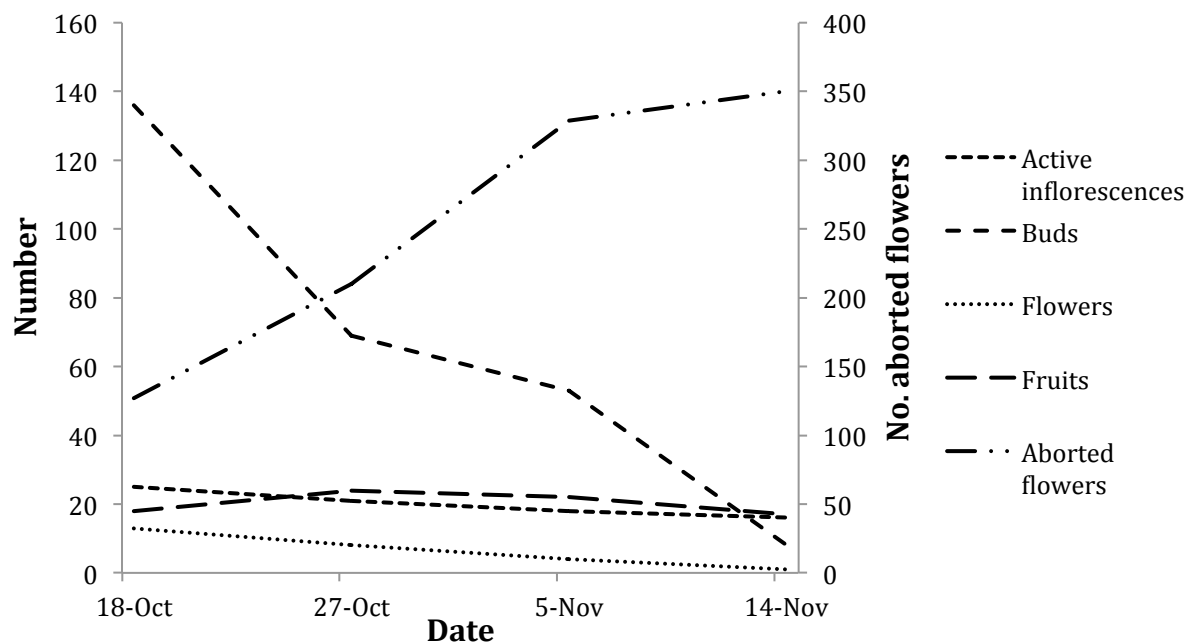


Figure 11. The phenology during the study period across the 26 study patches for *Vanilla bosseri* shown as the changing number of active inflorescences, buds, flowers and fruits (left axis), and the number of aborted flowers (right axis). All plants with active inflorescences, a total of 18 individuals, were included. Active inflorescences are defined as producing either buds, flowers or fruits during the time of the study.

### Floral morphology

A reproductive individual of *V. bosseri* carried on average 1.7 inflorescences (N=18, SD=1.2) with 15.3 flowers (N=32, SD=5.1). Flowering was successive with one flower open at a time. The flowers were ephemeral, open only from shortly after sunrise and wilting during the afternoon (Fig. 17). The length but not the width of the ovary varied much between individuals ( $43.7 \pm 9.5$  mm, CV=21.7% and  $3.5 \pm 0.2$  mm, CV=4.7%, respectively). Measurements of the floral morphology of fully expanded flowers are presented in Table 2. The display characters of the flower such as the width of the bright white sepals and petals were quite varied, as was the outer width of the labellum and the floral diameter (Table 2A). However, the labellum height and width at the column apex was relatively constant, as were the floral primary sexual structures that are important for the precision of the pollination mechanism (Table 2B-C). The inside of the large labellum was mainly white but gradually shifted into yellow further in (Fig. 12). The two-divided pink central crest and the very short dark orange tuft-like outgrowths between the crests both differed in colour from the bright white outer flower parts. The amount of this outgrowth varied considerably between individuals (Table 2A), which could indicate low selection pressure for size and shape. The central crests were positioned directly below the column apex, creating a passage for any insect trying to enter the innermost cavity of the flower (Fig. 12). The three-lobed stigma is emergent and flap-like, with two receptive lateral lobes covered completely by the sterile third lobe, which forms a broad, emergent rostellum (Fig. 13D). Sticky material is produced along the margins of the sterile lobe creating a diffuse viscidium. The pollen does not form true solid pollinia, instead it forms a somewhat ill-defined mass that is protected by an anther-cap (Fig. 13). For a human nose the open flower has a fairly constant but faint scent that is only perceived at a close distance. The scent is sweet, vanilla-like with a hint of cinnamon and honey. No indication of nectar or other exudate was found.



Figure 12. Longitudinal section of the labellum of *Vanilla bosseri*, with the left central crest removed to show the narrow passage under the column apex created by the central crest. Scale of small square on paper: 1 mm.



Table 2: Floral characteristics (mm) of *Vanilla bosseri* (one flower randomly selected from each of nine inflorescences). Measured characters are according to Fig. 8.

<b>Character</b>	<b>Mean</b>	<b>SD</b>	<b>CV (%)</b>	<b>Range</b>
<b>A. Display characters</b>				
Flower diameter (FD)	79.8	14.3	18.0	59.3-110.0
Lateral sepal				
Length (SL)	50.2	4.2	8.3	42.1-56.2
Width (SW)	17.3	2.3	13.5	12.7-21.3
Adaxial sepal				
Length (ASL)	49.3	4.2	8.4	45.0-55.9
Width (ASW)	15.0	3.0	19.7	10.9-19.3
Petal				
Length (PL)	50.6	4.0	7.9	44.9-58.3
Width (PW)	27.4	5.0	18.4	19.2-35.2
Labellum				
Length (LL)	43.5	3.5	8.1	37.9-49.7
Width (cut open) (LW)	31.6	5.0	16.0	24.2-40.1
Outer width (LOW)	15.7	3.7	23.7	10.0-19.1
Outer height (LOH)	18.8	1.7	9.2	17.1-21.7
Length of orange hair tuft (HL)	5.2	1.8	34.9	1.6-6.9
Height of orange hair tuft (HH)	1.1	0.3	25.7	0.8-1.7
<b>B. Mechanical characters</b>				
Labellum				
Inner width (LIW)	9.8	1.8	18.2	8.1-12.9
Inner height (LIH)	10.0	1.5	14.5	8.2-12.6
Width (at column) (LWC)	9.4	0.8	8.6	8.2-10.7
Height (at column) (LHC)	9.7	0.6	6.2	9.0-10.6
Labellum tip to central crest (CC-L)	18.1	2.8	15.5	13.7-21.7
Central crest				
Length (CCL)	12.4	1.5	12.2	10.1-14.6
Width (CCW)	4.0	1.1	27.5	1.7-5.7
Height (CCH)	1.5	0.3	18.8	1.1-2.0
Central crest to column distance (C-CC)	4.4	0.6	12.4	3.8-5.2
<b>C. Primary sex characters</b>				
Column				
Length (CL)	22.0	1.4	6.6	19.8-23.8
Anther				
Length (AL)	2.9	0.4	15.2	2.4-3.5
Width (AW)	2.5	0.3	13.2	2.0-2.9
Height (AH)	3.1	0.3	8.9	2.6-3.4
Rostellum				
Length (RL)	2.7	0.3	11.9	2.1-3.3
Width (RW)	2.0	0.3	14.1	1.7-2.5
Stigma				
Length	2.2	0.4	18.2	1.7-2.8
Width	1.2	0.0	3.4	1.2-1.3

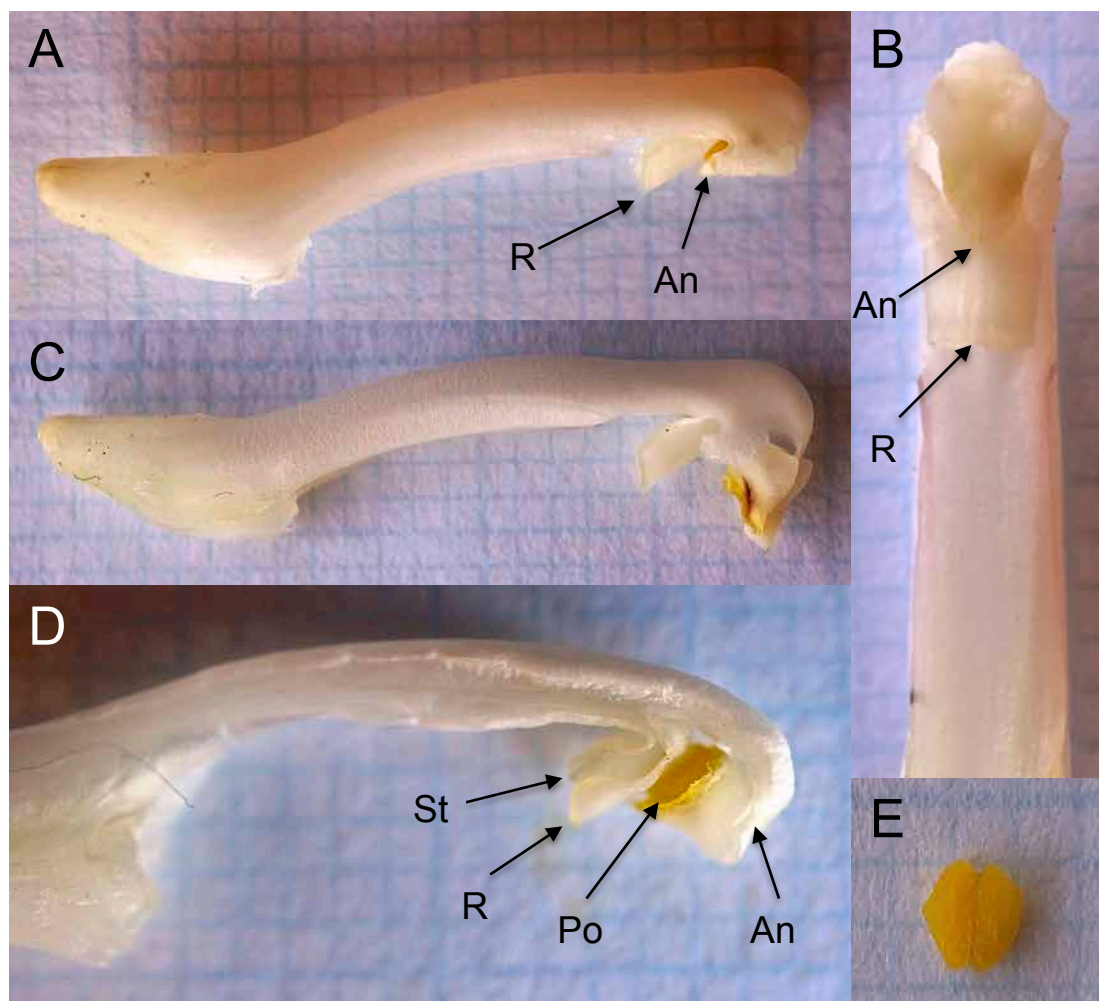


Figure 13. Column morphology of *Vanilla bosseri*. **A**: Intact column, lateral view. The distal edge of rostellum (R) and anther cap (An) are indicated by arrows. **B**: Intact column, ventral view. The distal edge of rostellum (R) and anther cap (An) are indicated by arrows. **C**: Column with the anther cap flipped open, lateral view. **D**: Longitudinal section of column shows the emergent receptive stigma (St), rostellum (R), and pollen (Po) inside the anther (An). **E**: Pollen-mass. – Scale of small square on paper: 1 mm.

### Floral visitors

According to field observations and voucher specimens the bee *Macrogalea ellioti* (Sauss.) was the most common floral visitor (Table 3), with a mean visiting frequency of 0.86 visits per hour. Of the total 54 observed flowers only one developed into a fruit, with the only observed visitor being *M. ellioti*. Also two species in the genus *Liotrigona* were frequent with a mean visiting frequency of 0.63 visits per hour. Because of the small size (2.5-3 mm) and superficial similarity between the *Liotrigona* species it was difficult to identify which of the two species that visited on each particular occasion and they are therefore merged in Table 3. In addition to these only one other visitor, a medium-sized greyish bee that most likely was *Lithurgus pullatus* Vach., entered the labellum and therefore had a potential to act as a pollinator. This bee had a very low visiting frequency and was never caught. The identification is therefore based on four observations, one of which was at a distance less than two meters and the other three with binoculars at a distance of about six meters. One of the longer-distant visits was also filmed.

Table 3. Flower visitors of *Vanilla bosseri* recorded in Kirindy forest, western Madagascar, in early November 2014. The mean duration of a flower visit and the time range for flower visits are presented for each species.

Visitor	No. visits	Mean duration (s)	Time range (s)
<b>Insecta</b>			
<b>Hymenoptera</b>			
Apidae			
<i>Macrogalea ellioti</i> (Sauss.) ♀	80	26 (N=30)	1-120
<i>Liotrigona madecassa</i> (Sauss.) w	58	324 (N=19)	10-1200
<i>Liotrigona mahafalya</i> B. & M. w			
Megachilidae			
<i>Lithurgus pullatus</i> Vach. *	4	9 (N=4)	1-30
Vespidae			
<i>Ropalidia</i> sp.*	5	40	-
Formicidae			
Unid. sp.	6	-	-
<b>Lepidoptera</b>			
Pieridae			
<i>Colotis evanthe</i> (Boisd.) ♂	2	4 (N=2)	2-6
Unid. sp. black	2	1	-
Unid. sp. small	1	7	-
<b>Orthoptera</b>			
Caelifera *	2	1800	-
<b>Diptera</b>			
Bombyliidae			
<i>Exoprosopa</i> spp. *	14	3 (N=7)	1-8
Unid. sp.	1	-	-
<b>Mammalia</b>			
<b>Primates</b>			
Indriidae			
<i>Propithecus verreauxi</i> (Grandidier)	1	-	-

\* Field identification only (insect not captured)

#### *Visitor behaviour*

Individual females of *Macrogalea ellioti* (Fig. 14) usually flew straight to the flower, hovering in front of the labellum for a few seconds before landing and then crawling into the labellum funnel. A visit typically lasted for about 20 seconds and was only once seen to last longer than a minute (Fig. 16). Whenever there were more than one flower open at the same patch the female typically visited all flowers and occasionally made revisits to the same flower. The behaviour of *M. ellioti* was one of constancy. The frequency of visits was highest between 8 and 10 a.m., a mismatch when compared to the flower anthesis, and no visits were seen later than 12 p.m. (Fig. 17). There was a general noticeable decrease in the total insect activity in the area after 12 p.m.

It proved difficult to observe the behaviour once the bee was inside the labellum because most of the flowers were situated more than 3 m up and for an observer were rather facing the sky. In the best observed cases, the bee crawled into the labellum and spent the majority of the time crawling on and around the central crest. Before leaving, it turned around inside the labellum and left head first. No direct field observations could be made on mechanical interactions between the bee and the anther or stigma. Two individuals of *M. ellioti* were caught when leaving a flower and both were carrying sticky pollen similar to that of *V. bosseri* on the tibia of the hind



legs. One of these individuals also carried pollen from a non-orchid flower and had a pollen deposit from *V. bosseri* on the fore part of the mesonotum (Fig. 14).



Figure 14. Female of *Macrogalea ellioti* that was caught when leaving a *Vanilla bosseri* flower. Arrows indicating pollen collected on hind tibia and pollen on the anterior part of mesonotum. Scale: 5 mm.

Workers of *Liotrigona madecassa* (Sauss.) and *L. mahafalya* B. & M. (Fig. 15) were not only common visitors on open flowers but also frequently crawled on wilted ones and buds. The length of visits varied very much, from a few seconds up to 20 minutes (Fig. 16), as was the frequency of visits during the day with a peak in the early morning that does not correspond with the flower anthesis (Fig. 17). A visit was typically not limited to certain parts of the flower, the individual usually crawled both on petals and sepals, as well as on the inside and outside of the labellum. Several observations were made of direct contact with the anther, with the individual searching the anther and possibly collecting pollen. Two individuals of *L. madecassa* and one of *L. mahafalya* were caught when leaving the flower. The latter and one of the former were carrying pollen similar to that of *V. bosseri* on the tibia of the hind legs (Fig. 15 right).



Figure 15. *Liotrigona mahafalya* (left) and *L. madecassa* (right) workers caught when leaving *Vanilla bosseri* flowers. Pollen collected on hind tibia indicated with an arrow on *L. madecassa*. Note that *L. madecassa* is considerably smaller than *L. mahafalya*. Scale: 1 mm.

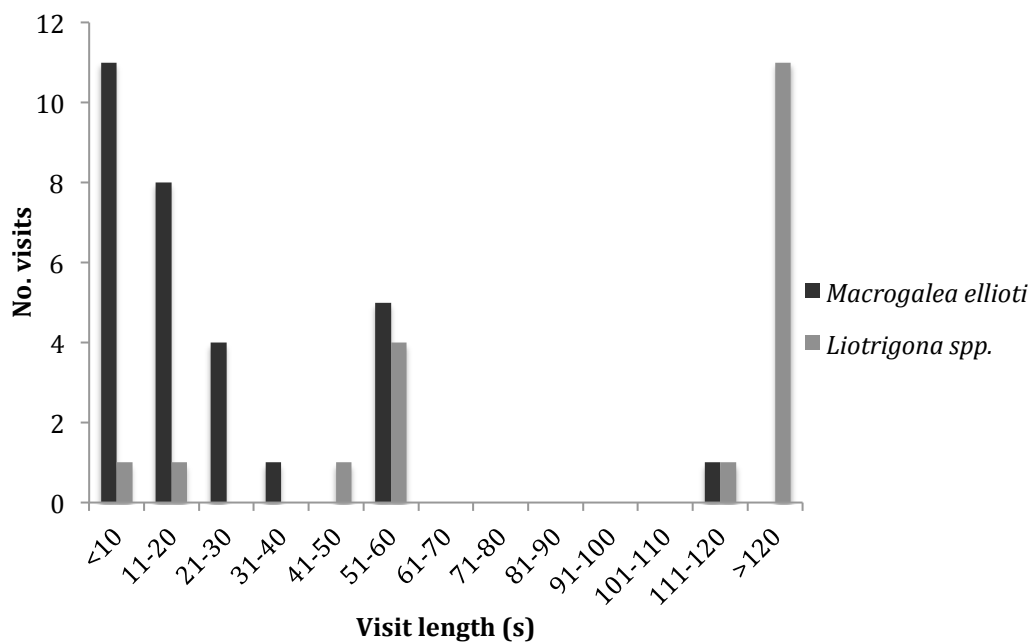


Figure 16. Frequency distribution of visits to *Vanilla bosseri* based on visit duration for the most common visitors (*Macrogalea ellioti*, *Liotrigona madecassa* and *L. mahafalya*).

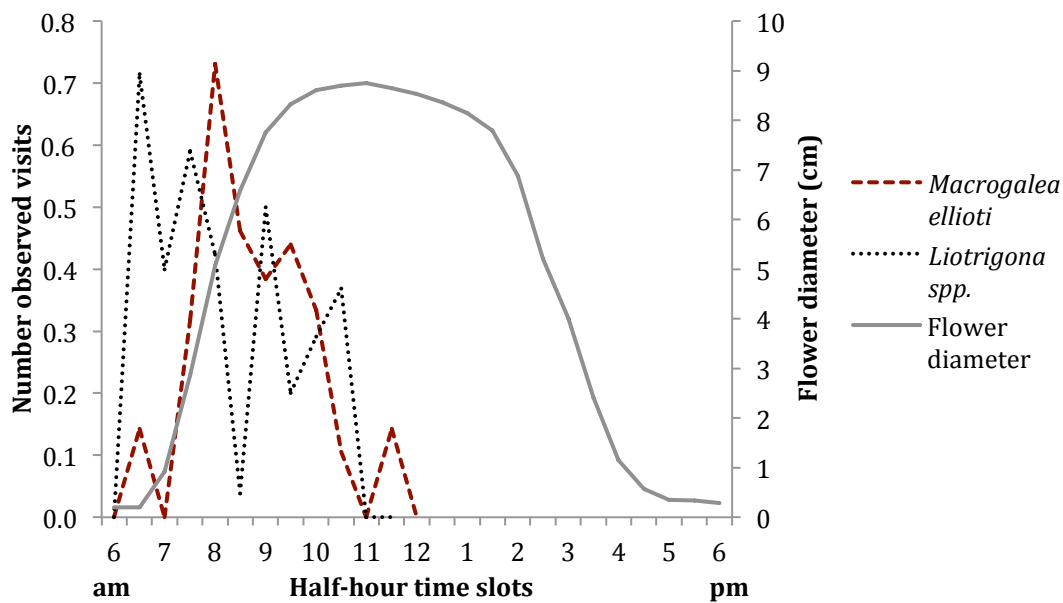


Figure 17. Total number observed visits to the flowers of *Vanilla bosseri* per half hour time slots by the most frequent bee visitors (*Macrogalea ellioti* and *Liotrigona* spp.) The number of visits has been scaled against the number of hours observed per time slot. The floral diameter corresponds to the flower anthesis.

A bee tentatively identified as *Lithurgus pullatus* was observed visiting *V. bosseri* at four occasions. It flew straight to the flower, hovering for a fraction of a second before landing and then entered the labellum directly. When inside, the bee's body filled up the labellum completely. It left the flower by backing out of the labellum and then rapidly left the flower. At the sites where *L. pullatus* was observed only one inflorescence with a single open flower was present. Thus if constancy was practised it was not possible to record. Three of the visits were very short and lasted for only about two seconds, but one lasted for 30 seconds.

Wasps tentatively identified as *Ropalidia* sp. was observed to visit *V. bosseri* on five occasions. The wasps were clearly attracted to the flower as they flew straight toward it. However, they never entered the labellum, but instead crawled on the sepals and petals, as if exploring, before leaving the patch. One small, black unidentified species of ant (Formicidae) was frequently observed crawling along the vines and inflorescence of *V. bosseri*. At six occasions individuals also crawled on the open flower, but only entered the labellum once. Three Lepidoptera species were attracted to and visited *V. bosseri*. None of them tried to enter the labellum, for which they would have been too large, instead they all landed on the petals as well as on still white wilted flowers. Two Caelifera species were observed on open *V. bosseri* flowers. On both occasions they sat on petals or the outside of the labellum and seemed to be feeding on the flower tissue. Both species were much too large to be able to enter the labellum. Bee flies of the genus *Exoprosopa* spp. were observed visiting *V. bosseri* on 14 occasions. All species were too large and awkward to enter the labellum. During a typical visit the visitor hovered close to the opening of labellum, only landing for a few seconds on the outer part before leaving the flower. Verreaux's sifaka, *Propithecus verreauxi*, which is the largest lemur species in the area, was on one occasion seen feeding on flowers of *V. bosseri* in a clearly

destructive manner. All of these non-bee visitors are considered “ecological noise”, as they for different reasons cannot act as efficient pollinators.

### *Bee morphology*

Morphological measurements of the bees caught leaving *V. bosseri* flowers are presented in Table 4. In addition, measurements of some further typical species of the local bee fauna in Kirindy forest in November are also included.

Table 4. Approximate operative sizes (mm) of different bees for comparison with the flower morphology of *Vanilla bosseri* at Kirindy forest. w = worker. ♀ = female. \*Bees observed and collected on other flowers in Kirindy forest 22-27 November 1989 by L.A. Nilsson.

<b>Bee species</b>	<b>Thorax height</b>	<b>Thorax Length</b>	<b>Functional height</b>	<b>Body length</b>
<b>Visiting <i>V. bosseri</i></b>				
<i>Macrogalea ellioti</i> (Sauss.) ♀	2	2	<b>3</b>	9
<i>Liotrigona madecassa</i> (Sauss.) w	1	1	<b>1.5</b>	2.5
<i>Liotrigona mahafalya</i> B. & M. w	1	1.5	<b>1.5</b>	3
<b>Others*</b>				
<i>Lasioglossum moderatum</i> (Ben.) ♀	1.5	2	<b>2</b>	6
<i>Lithurgus pullatus</i> Vach. ♀	3	3.5	<b>4</b>	12.5
<i>Megachile albiscopa</i> Sauss. ♀	3	3	<b>4</b>	10
<i>Megachile brooksi</i> Pauly ♀	3	3	<b>4</b>	11
<i>Megachile submetallica</i> Ben. ♀	4	3.5	<b>5.5</b>	13
<i>Amegilla antimena</i> (Sauss.) ♀	4	4	<b>5.5</b>	11
<i>Tetraloniella madecassa</i> (Ben.) ♀	3.5	3.5	<b>4.5</b>	11
<i>Apis mellifera unicolor</i> Latr. w	4	4	<b>5.5</b>	11.5

### **Pollination mechanism**

Based on the floral morphology and the morphology and behaviour of bees a rather simple pollination mechanism can be described for *V. bosseri*. The flower forms a distally open chamber that a bee must enter; the cavity is created by the labellum that is attached to the column margins. The pollination mechanism is activated when the bee leaves the chamber. The central crest creates an obstacle that the bee must climb over. When doing so the bee lifts its body and first passes by the stigma. Thereafter the bee brushes against the lower part of the emergent rostellum that supplies sticky viscid material, which subsequently helps to pick up pollen from the anther. This pollen can then be deposited on the emergent stigma in a later visited flower.

### **Breeding system**

The natural fruit set for *V. bosseri* across the 26 patches was 3.96% (17 out of 429 flowers). In the pollination experiment the cross-pollination and self-pollination treatments had fruit sets of 43% and 86%, respectively. These are both significantly higher than the natural fruit set (Fisher’s exact test,  $P < 0.003$  and  $P < 0.001$ ). The self-pollination treatment had a higher fruit set than the cross-pollination one, but this was not significant (Fisher’s exact test,  $P = 0.27$ ). The fruit set for the caged treatment was 0%, with no tendency of swelling before the flower fell off.

## Discussion

### Breeding system

The pollination experiment indicates that *V. bosseri* is allogamous but self-compatible. The resulting fruit set for self-pollinated flowers was twice as high as for the cross-pollinated flowers, a however non-significant result. Most allogamous orchids produce higher fruit sets when cross-pollinated (Tremblay *et al.* 2005). The experiment makes it difficult to draw any conclusion about a possible reverse result but rather that larger sample sizes, preferably including individuals from several sites, are needed. The very low natural fruit set (3.96%), combined with the 0% fruit set for the caged flower treatment, strongly indicates that *V. bosseri* is not autogamous. In comparison, the natural fruit set of *V. humblotii* has been mentioned to be <1% (Gigant *et al.* 2014) and around 1% in *V. madagascariensis* (Perrier de la Bâthie 1934). Spontaneous self-pollination is uncommon for most *Vanilla* species, most likely due to the large rostellum that has an important function in pollen dispersal and, as a consequence, effectively prevents contact between the stigma and stamen (Bory *et al.* 2008). Autogamy has, however, been suggested to occur for a few *Vanilla* species, all of which have unusually high natural fruit sets (Householder *et al.* 2010, Soto Arenas and Dressler 2010, Van Dam *et al.* 2010).

There was a more than tenfold difference between fruit set for the hand-pollinated flowers and the natural fruit set. This indicates that the system is pollen limited, a common characteristic for non-autogamous orchids (Tremblay *et al.* 2005). Moreover, pollen limitation in *V. bosseri* is probably strong. Tremblay *et al.* (2005) describe three causes for pollen limitation, (1) low pollinator abundance, resulting in some flowers never being visited, (2) low amount of pollen reaching the stigma, even when pollinator visits are frequent, and (3) low pollen quality. The present study has confirmed a low visiting frequency of bees that pollinate *V. bosseri*, a level that may largely explain the pollen limitation observed. That *V. bosseri* does not form true pollinia may to some extent limit the pollen loss. It has been showed that pollen removal to fruit set ratio is more efficient for orchid species with mealy pollen that can be broken into submasses than those that form solid pollinia (Tremblay *et al.* 2005).

### Legitimate pollinator and pollination system

The flower-visitors and the floral morphology of *V. bosseri* clearly indicate that the flower is bee pollinated. This is consistent with the present knowledge of pollinators for other *Vanilla* species, all of which are pollinated by different bee species and genera (Ackerman 1983, Roubik and Ackerman 1987, Soto Arenas and Cameron 2003, Soto Arenas and Dressler 2010, Gigant *et al.* 2014). Furthermore Rasmussen (1985) interpreted the pollination act in the genus as follows “The flower is gullet-shaped and often provided with a tuft of retrorse hairs or crests, forcing visiting insects, when retreating, to raise their body and thereby touch the versatile anther”. Judged from the floral morphology an effective pollinator of *V. bosseri* would be a bee that is of a certain size, especially since the central-crest-to-column distance measured  $4.4 \pm 0.6$  mm. An effective pollinator is expected to have a functional height that is comparable to be able to efficiently activate the pollination mechanism. This indicates that the effective pollinator is smaller than typical medium sized common bees in Kirindy such as the honeybee *Apis mellifera unicolor*, *Amegilla antimena* and *Megachile submetallica* that all have a functional height of ca 5.5 mm.

On the other hand the functional height of *Lasioglossum moderatum*, 2 mm, is too small to activate the pollination mechanism of *V. bosseri*, as are the two commonly observed tiny visitors *Liotrigona mahafalya* and *L. madecassa* with a functional height of only 1.5 mm. Neither of these three small bee species are likely to lift their bodies enough to be an effective pollinator. The *Liotrigona* specimens caught carrying pollen similar to that of *V. bosseri* and the direct observations of interaction and probable pollen collection from the anther suggest that they instead might act as pollen thieves. Such behaviour may result in pollen limitation and have been showed to lower the reproductive success of the plant *Aloe maculate* (Hargreaves 2007).

In relation to the flower morphology of *V. bosseri* medium-sized bees such as *Lithurgus pullatus*, *Tetraloniella madecassa* and several others all have a functional height (4-4.5 mm) that would allow them to act as an effective pollinator. Indeed, (tentative) *L. pullatus* was observed visiting *V. bosseri*. The distribution of this species in western Madagascar corresponds fairly well with to that known for *V. bosseri* (Pauly *et al.* 2001). *T. madecassa*, that morphologically is the best fit with a functional height of 4.5 mm, only occurs in dry habitats that might also be suitable for *V. bosseri*. It has a similar distribution in the south of Madagascar as *L. pullatus* (Pauly *et al.* 2001), and has been observed in Ankarafantsika, near Majunga (L.A. Nilsson pers. comm.), the other known locality for *V. bosseri*. There is reason to believe that *V. bosseri* is more widespread than the two known localities since the species was only recently described and is easily confused with the two other white Malagasy *Vanilla* species, *V. decaryana* and *V. madagascariensis*. Both these are distributed in dry forest along Madagascar's west coast (Cribb and Hermans 2009), which is also where additional localities of *V. bosseri* might be found.

The most common visitor, females of *Macrogalea ellioti*, has a functional height of ca 3 mm. This is probably a little too small for an effective pollinator, as the bee would have to lift its body more than 1 mm to frequently and successfully activate the pollination mechanism. Nevertheless the pollen deposit on the mesonotum of one individual caught shows that contact occurs at least sometimes, and the pollen placement is consistent with the observations that *M. ellioti* leaves the labellum head first. It is not known how the bee turns around inside the labellum cavity. Possibly the bee performs a somersault. Because the bee's movement within the labellum remains unknown it is not known how exactly *M. ellioti* interacts with the rostellum and anther or deposits pollen on the stigma. *M. ellioti* occurs in dry forests all along Madagascar's west coast (Pauly *et al.* 2001), a distribution that corresponds to the area where additional localities of *V. bosseri* might be found.

The floral morphology and lack of nectar suggest that *Vanilla bosseri* has evolved to act in deceit in relation to its pollinators and that the flower is a generalized food-flower mimic. Generalized food deceptive systems are characterized by a lack of reward, short duration of floral visits and few consecutive visits. It is a common strategy for orchids with low population densities (Ackerman 1986). Deceptive systems have been described for *V. planifolia*, *V. odorata*, *V. insignis* and *V. roscheri* (Soto Arenas 1999, Gigant *et al.* 2014). The short flower visits by *L. pullatus* are typical for food deceptive systems. However, the behaviour of *M. ellioti* does not match to that of a deceived pollinator. Most *M. ellioti* visits to *V. bosseri* were quite long and the females' consistent behaviour of visiting all open flowers and even revisiting flowers at a patch further suggests that they did not experience deceit, but

rather reward. Both specimens of *M. ellioti* caught were carrying pollen loads on their hind tibiae with pollen similar to that of *V. bosseri*. This would indicate that they were collecting pollen for brood provisioning, a behaviour that has been suggested for other allodapine bees when visiting the African species *Vanilla roscheri* (Gigant *et al.* 2014). The visits of *M. ellioti* usually lasted for about 20 seconds, which is considerably shorter than visits by the common visitors *Liotrigona* spp., but may be sufficient for pollen collection. The difference in visit duration most likely reflects the biology of the species, where social *Liotrigona* spp. are opportunists compared with the solitary *M. ellioti*. The observations would then indicate that *M. ellioti* gets a pollen reward from the visits, thereby most likely cheating the main pollination mechanism of the flower. Pollen collection would make at least part of the pollen unavailable for pollination, suggesting *M. ellioti* might rather act as a pollen thief and reduce the reproductive success of the orchid. Allodapine bees that collect pollen have been shown to cause a reduction in reproductive success of *Aloe maculata* (Aloeaceae) due to enforced pollen limitation (Hargreaves *et al.* 2010).

That pollen-collecting insects act as orchid pollinators are known from a few cases. Interestingly, such pollination systems have been described for several primitive orchid genera. *Apostasia* is believed to be pollinated by buzzing bees and *Trigona* bees have been observed collecting pollen presented on the labellum and scrape pollen from the anther of *Neuwiedia* (Vogel 1981, Kocyan and Endress 2001). *Cleisthes* is pollinated by bumblebees (*Bombus*) that groom away some of the pollen deposited on the dorsal thorax and pack it into their hind tibial corbiculae (Gregg 1991). The mechanism of pollination and pollen collection differs for these three orchid genera, but an important trait they share is mealy and soft pollen-masses, a trait also present in the *Vanilla* genus. Allodapine bees (*Allodapula variegata* and *Allodape rufogastra*) have been suggested to act as pollen-collecting pollinators for the African species *Vanilla roscheri* (Gigant *et al.* 2014). As far as can be judged from the Illustrations, that species is morphologically very similar to *V. bosseri*. Based on observed pollen movement these authors presented two different pollination mechanisms depending on the attracted pollinator. The first, and most likely the legitimate, pollination system is a generalized food deceptive system with anthophorine bees acting as the main pollinators with pollen deposits on the mesonotum. The second is a reward system that attracts pollinators such as allodapine bees with pollen as the reward (Gigant *et al.* 2014). The observations in the present study are very similar to those described for *V. roscheri*. That only one of the 54 observed flowers developed into fruit, with the only observed visitor being *M. ellioti*, provides rather strong evidence that *M. ellioti* is very inefficient as a pollinator. The data suggest that *V. bosseri* use a generalized food deceptive system but perhaps the primary pollinator is not yet revealed. The main observed visitor *M. ellioti* is most likely a pollen thief that only occasionally acts as a pollinator.

### **Attraction of pollinators**

At long distances bees are often attracted to flowers by optical cues, such as colour and size (Proctor *et al.* 1996, Giurfa and Lehrer 2001). *V. bosseri* blooms during the dry season when few species have developed leaves and the large, bright white flower is therefore conspicuous within the forest. Most flowers that are perceived as white by humans do not reflect ultra-violet, and bees therefore see them as coloured (Proctor *et al.* 1996). The orange-yellow inner labellum creates a colour contrast from the rest of the flower that likely contributes to the close up orientation of the pollinator. False

nectar guides such as spots and lines can stimulate visiting and food-searching reactions by potential pollinators (Nilsson 1980). It is possible that the dark orange tuft in the bottom of the labellum contribute to the flower attraction in a similar manner. Flower fragrance has been shown to be more important than visual cues for bees when orientating on short distances to adjust their approach and landing (Free 1970). The *Vanilla* genus is known for its fragrant flowers, although there are non-fragrant species, and the fragrance seems to play an important part in attracting pollinators for many *Vanilla* species (Soto Arenas and Cameron 2003, Householder *et al.* 2010, Soto Arenas and Dressler 2010). For example, the unusual high fruit set produced by *V. chamissonis* Klotzsch is attributed to the strong floral fragrance (Gigant *et al.* 2011). Also, Householder *et al.* (2010) described how particularly fragrant flowers of *V. pompona* (Lindl.) *grandiflora* Soto Arenas attract a greater number of pollinators than individual flowers with faint fragrance. To humans, the faint fragrance of *V. bosseri* is only perceptible at a close distance. Most likely the pollinating bees are initially attracted to the flowers of *V. bosseri* because of its conspicuous size, colour and placement in the canopy. The close up orientation of the bee pollinator is thereafter likely guided by a combination of fragrance, colour and finally the labellum cavity itself.

Several deceptive orchids are known to attract insect pollinators by mimicking rewarding flowers in the surrounding environment (Dafni 1984). The *Uncarina* species seen in the area have large funnel shaped yellow flowers principally similar to the flowers of *V. bosseri*. Both *L. pullatus* and *M. ellioti* have been observed visiting the yellow *Uncarina* sp. in Kirindy forest (L.A. Nilsson pers. comm.). *M. ellioti* displayed the same behaviour of leaving the flower head first, suggesting similar movements within the cavity of the flowers of both plant species. Interestingly the distribution and habitat of the only white *Uncarina* species, *U. leptocarpa* (Decane.) Ihlenf. & Straka, is similar to that of *V. bosseri* (Humbert 1971, Eggli 2002), and it has been reported to occur in the dry forest northeast of Morondava (Tropicos 2015), even though it was not seen during this study. The resemblance in floral morphology and shared bee visitors might suggest that *V. bosseri* has evolved as a mimic of the rewarding *Uncarina*. However, the possible interrelation between the two plants is uncertain and requires further study. Also, the *Uncarina* species seemed to be scarce in the forest and not observed in the vicinity of *V. bosseri*. The magnet-species theory suggests that non-rewarding plants can benefit from growing in close proximity to rewarding flowers (Thomson 1978, Johnson *et al.* 2003). But it is improbable that *V. bosseri* attracts additional pollinators that way, as it was not seen to grow in vicinity of other flowering plants. Generally other flowering species were scarce in the forest, which might instead benefit *V. bosseri* as it minimizes the competition for pollinators from reward producing plants.

### **Conservation aspects**

Madagascar is one of the world's hot spots for biodiversity when species endemism and degree of threat are considered (Myers *et al.* 2000). The high level of endemism is due to the islands long isolation, as it has been a separate landmass for at least 88 million years (Ganzhorn *et al.* 2014). Eleven endemic plant families, 310 endemic genera of plants and more than 9000 endemic plant species can be found here, which constitutes 3.2% of the world's total 300 000 plant species (Myers *et al.* 2000). The island's orchid flora is rich, with approximately 1000 species in 59 genera, of which more than 88% of the species and 12 of the genera are considered to be endemic (Du



Puy *et al.* 1999). Though only generalized information on distribution and ecology is known for most orchid species, many are considered threatened, particularly by habitat destruction (Cribb *et al.* 2005). Madagascar has experienced a rapid habitat loss with only 9.9% of the primary vegetation remaining by 1999 (Myers *et al.* 2000). The majority of the island constitutes very species-poor secondary grasslands and most areas are burnt annually and exposed to intense erosion (Du Puy and Moat 1998). The seasonally dry, deciduous forest occurs mainly in the west and south of Madagascar (Cribb *et al.* 2005), but is a habitat type that remains inadequately protected (Du Puy and Moat 1998). There is no available information about the conservation status of *V. bosseri*, or any other Malagasy *Vanilla* species, but there is reason to believe that without further protection of their habitat the species will go extinct. The leafless *Vanilla* species are under additional pressure as local people collect the vines for medicinal purposes (Cribb *et al.* 2005). This study has contributed to the understanding of the reproductive biology of *V. bosseri*, a crucial life history part in any future conservation of this endemic species.

## Conclusion

The breeding experiments indicate that *V. bosseri* is allogamous but self-compatible and dependent on pollinators for reproduction. Flower visitors, floral morphology and lack of nectar suggest that it is a bee-pollinated generalized food-flower mimic. The pollination mechanism is rather straightforward. When leaving the flower the bee passes the central crest and is forced to raise its body, thus activating the pollination mechanism by coming into contact with the rostellum before the anther and later on the stigma in another flower. However, the data suggest that the main visitor, the allodapine bee *Macrogalea ellioti*, is a pollen thief that only occasionally acts as a pollinator. Further studies, preferably earlier during the flowering season, are needed to reveal if there is a more efficient pollinator. One may predict from the floral morphology that an effective pollinator would have an operative size similar to that of *Lithurgus pullatus* or *Tetraloniella madecassa*. This study has paved the way to the detailed knowledge about the reproductive biology of this endemic orchid species within its highly threatened habitat.

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