

Structural specialisation and pollination ecology in two Malagasy species of *Dalechampia* (Euphorbiaceae)

Olive Imanizabayo, Dian Fossey Gorilla Fund International, Rwanda

Marco Plebani, University of Zurich, Switzerland

Abstract

We compared structural features of the blossoms and aspects of pollination ecology for the Madagascan endemic vines *Dalechampia bernieri* and *D. subternata*, characterised by specialised and generalised pollination, respectively. We measured bract aperture and length, visitation rate of the blossoms and the amount of pollen on female flowers. As expected, *D. subternata*'s bract angle was stable during the day while *D. bernieri*'s bract angles changed daily, becoming more widely open when its specific buzz-pollinating bees were active. Contrary to expectations, *D. subternata* flowers were minimally pollinated, while the amount of pollen on *D. bernieri*'s flowers decreased when they were visited by specialist pollinators together with more opportunistic ones.

INTRODUCTION

Dalechampia L. (Euphorbiaceae) is a pan-tropical genus of vines and shrubs represented by over 120 species. It is characterised by typical inflorescences called pseudanthia (hereafter "blossoms"), in which male and female flowers are organised in a unit functionally equivalent to the stili and stigmata of a monoecious flower. The genus has drawn attention for the unusual rewards offered to its pollinators as well as for its complex evolutionary history.

The *Dalechampia* genus evolved in the Neotropics from an ancestor offering fragrances and/or pollen as a reward to pollinators. Most of the genus's modern species in the Neotropics and in continental Africa offer resin to its pollinators: this non-edible reward is collected by large bees of the families Megachilidae and Apiidae, which use it for the hive construction. A few *Dalechampia* species offer monoterpenic fragrances as a reward, which are collected by male euglossine bees and used as a precursor of sexual pheromones.

A consequence of these unusual rewards is the high specificity of the pollinators: most *Dalechampia* species are pollinated by only one or two species of bees, and on the African mainland, *Dalechampia* populations are usually pollinated by only one species of *Pachyanthidium* or *Heriades* bees (Megachilidae; Armbruster & Steiner, 1992).

The genus *Dalechampia* has colonised Madagascar in relatively recent times and it is represented there by 10-12 endemic species. In contrast to most of the co-generic species in the Neotropics and in the

African mainland, Malagasy species do not offer any reward to the pollinators apart from pollen itself. This is considered a consequence of the lack of Megachiliid and Apiid bees on the island: in such a situation, evolutionary pressure led *Dalechampia* species to de-specialise and switch back to a less advantageous but more widely accepted form of reward.

An example of this evolution toward generalisation is *Dalechampia subternata*: this species has open male flowers and large bracts widely open facing upwards. These adaptations facilitate the access to the pollen for a broad range of generalist pollinators including bees, flies and beetles. A possible drawback of generalist pollination for pollen-rewarding plants is the high cost of producing large amounts of pollen that can go largely wasted, either used by the pollinators or dispersed on non-target flowers. Perhaps in response to this evolutionary pressure, some Malagasy species have gone through processes of secondary specialisation.

The main object of the present study, *Dalechampia bernieri*, represents a good example of this phenomenon. *D. bernieri*'s mature male flowers do not open as the ones of *D. subternata*: instead, a pedomorphosis process leads them to remain partially closed. The particular structure of *D. bernieri*'s male flowers may have evolved to allow access to the pollen specifically to bees capable of buzz pollination. Vibratile or 'buzz' pollination is operated by bees that have the ability to vibrate their thoracic muscles in order to extract the pollen from the stamina by a sonication process. Buzz pollination on *D. bernieri*'s male flowers operated by *Xylocopa* bees has been observed near Ramena, Antsiranana, in January 1996 (S. Armbruster, pers. comm.).

The purpose of the present study is to assess the structural and functional specialisation of *D. bernieri* compared to *D. subternata*, known to be pollinated aspecifically.

In particular, we aim to test the following hypotheses:

- 1) Bract aperture in *D. bernieri* changes during the day, increasing as the specific pollinators become more active and decreasing when they reduce activity, in order to protect the blossom's flowers from damages or non-specific pollinators. On the contrary, bract aperture in *D. subternata* is not expected to change significantly nor systematically at different times of the day.
- 2) We expect the structural specialisation of *D. bernieri* to be matched by ecological specialisation: in other words, we expect *D. bernieri* to be prevalently visited by buzz-pollinating bees, while we expect *D. subternata* to be visited by a larger and more heterogeneous set of pollinators.

- 3) We expect the more specialised species to be pollinated more efficiently. Practically, this would translate in a larger amount of pollen grains on the stigmata of *D. bernieri*'s blossoms compared to *D. subternata*'s blossoms, and in a larger amount of pollen grains on the stigmata of *D. bernieri*'s blossoms visited by buzz-pollinators compared to *D. subternata*'s blossoms which are mainly visited by less specific pollinators.



Figure 1. Blossoms of *D. subternata* (left) and *D. bernieri* (right). Note a *Liotrigona* bee on *D. bernieri* (see text for details).

MATERIAL AND METHODS

The study was conducted in the Kirindy Natural Reserve, central-western Madagascar (20.06717 °S 44.67709 °E). Kirindy represents one of the few remnant patches of coastal deciduous forest in Madagascar. The area of Kirindy currently protected as a Natural Reserve used to be subject to selective logging: as a result, Kirindy presents a gradient of grids ranging from pristine forest to patches where trees of specific diameters were selectively removed.

The data collection was carried out during six days from November 14th to November 19th 2012 (days 1 and 6 of the study, respectively). On the first day of the study, two vines of *Dalechampia subternata* (A and B) and nine vines of *Dalechampia bernieri* (A to L; letter I was not used) were surveyed and identified alphabetically. On each of the plants, 1-12 blossoms were individually marked. The choice of the plants was not performed randomly, but depended largely on logistic constraints (presence of blossoms). All the plants but one were found along Conoco road, the main road running through Kirindy. The study area spanned across a linear distance of approximately 5 km. Only *D. bernieri* L was located away from Conoco road's side, about 30 metres into an unlogged patch of forest.

For each blossom, the angle between the bracts was recorded every day at different moments of the day, 1-4 times per day. The angles were assessed visually by the same person during the whole study.

On day 5 of the study, the length of the bracts of each blossom was recorded using analogue callipers.

The presence of pollen on each female flower was assessed on days 2 to 5 of the study. We surveyed the blossoms at least once a day by means of a x10 hand-lens. The amount of pollen on each stigma was measured in a semi-quantitative way. Each stigma was ranked following a 5-levels scale, in which "0" means that the pollen is absent, "1" indicates the presence of approximately 1-5 pollen grains, "2" ~ 6-10 pollen grains, "3" ~ 11-25 pollen grains and "4" indicates the presence of >25 pollen grains, usually so many to be easily seen by the bare eye.

The identity and abundance of pollinators on *D. bernieri* and *D. subternata* was assessed by direct observation. The presence of pollinators was accounted for all plants during the surveys aimed at measuring pollen abundance and bract angles. These observations usually lasted only < 10 minutes. More extensive surveys, lasting one to four hours each, were conducted on *D. bernieri* C, D and N (selected because they showed high abundance of blossoms) and on *D. subternata* A and B (representing our entire sample for this species). Manpower constraints did not allow us to observe all the above mentioned plants daily and at the same time; the surveys were therefore performed asystematically, but they were mainly concentrated in the mornings, when the insect activity was observed to be particularly high.

The observed pollinators were identified at the lowest possible taxonomic level based on pictures or captured specimens.

Visitation rates were calculated for each pollinator species by summing the number of visits observed and dividing by the number of inflorescences open in the observation area during the period of observation. When possible, visitation rates for specific blossoms (i.e. the marked ones) were also calculated.

Analysis

The effect of species identity and moment of the day on changes in the bract aperture was assessed graphically and statistically.

The effect of different days on the bract aperture was assumed to be negligible on a time scale as short as the one of our study; therefore, observations taken on different days were pooled to gain a better coverage of a 24-hour time span.

We fitted a general linear model to the data, allowing for a polynomial dependence of the bract aperture on the time of the day. For *D. bernieri*, a piecewise regression was also performed to estimate the pace at which the bract angles increase or decrease in different times of the day.

The effect of days and location on the variation of the total pollen load on female flowers of *D. bernieri* C, D and N was assessed by fitting a general linear model (ANCOVA) to the data. This analysis was conceived as an indirect test of the effect of buzz-pollinators on the efficiency of pollination. In these analyses, data for *D. bernieri* C and D were pooled as the two vines were entangled and hard to distinguish separately.

All analyses were performed using MINITAB Release 13.32 (2000 Minitab Inc.) and R version 2.15.1 (2012).

RESULTS

The change of bract angles during the day showed a strong effect of species identity. Bract aperture in *Dalechampia subternata* was not affected by the moment of the day when they were measured (Figure 1, left); conversely, the time of the measurement had a strong non-linear effect on the bract aperture of *Dalechampia bernieri* (Figure 2, right). In *D. bernieri*, the blossoms increased linearly their aperture during the morning reaching a maximum around 11.00-12.00; after this moment, the blossoms progressively reduced the bract aperture in a linear fashion, until reaching the minimum at around 23.00-24.00 (Figure 3).

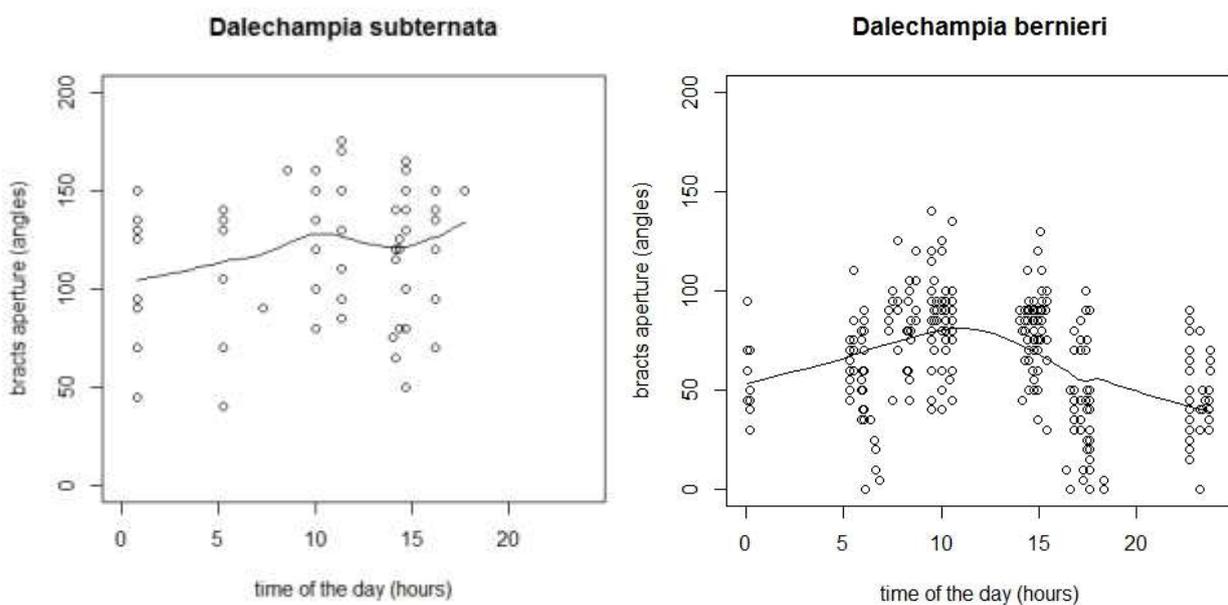


Figure 2. Changes in the bract angle for *Dalechampia subternata* (left) and *Dalechampia bernieri* (right). The curve line is the result of local polynomial regression fits.

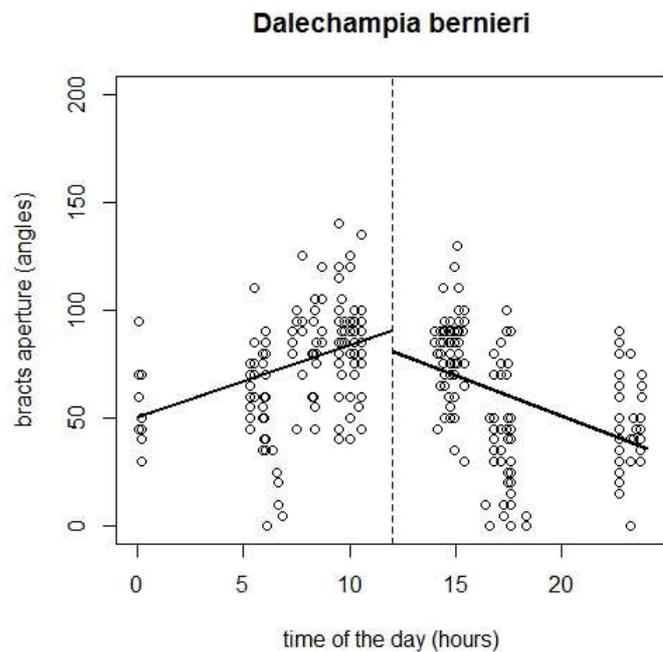


Figure 3. Segmented regression on *D. bernieri*'s angle measures. Slope for the left-hand line: 3.3407 ($p \ll 0.0001$). Slope for the right-hand line: -3.7532 ($p \ll 0.0001$). The dotted line corresponds to 12.00.

The results of the observation of pollinator visits are summarised in Table 1. There were striking differences between the two *Dalechampia* species. Almost no insects were observed on *D. subternata*'s blossoms, with the exception of a beetle and a few ants. *D. bernieri* was notable for a much higher visitation rate by pollinators, mainly stingless bees of the genus *Liotrigona* and *Nomia viridilimbata* bees, the latter capable of buzz-pollination (personal observation of the authors, who could also discern the “buzzing” sound produced by a visiting bee). A strong effect of location on the visitation rate by different pollinators was noticed between *D. bernieri* plants, as *Nomia viridilimbata* bees were active only on *D. bernieri* N.

Table 1. Visitation rates for the potential pollinators observed on the plants in study. *D. subternata* A and B were analysed together, as well as *D. bernieri* C and D. Visitation rates are expressed as mean number of visits per blossom per hour +/- SD. Single visits recorded over short observation times are not indicated as rates, but as absolute numbers.

| Plant species | Plant ID | Visitors group | | | |
|----------------------|----------|----------------|----------------------------|------------------------|------|
| | | Beetles | <i>Nomia viridilimbata</i> | <i>Liotrigona</i> spp. | Ants |
| <i>D. subternata</i> | A & B | 1 | 0 | 0 | 2 |
| <i>D. bernieri</i> | C & D | 0 | 0 | 14,64 | 0 |
| | E | 1 observation | 0 | 0 | 0 |
| | N | 1 observation | 4.07+-3.14 | 1.04+-0.70 | 0 |
| | M | 1 observation | 0 | 0 | 0 |

The variation in pollen accumulation was not significant across different days for plants C and D, while the amount of pollen on the blossoms of *D. bernieri* N decreased significantly during the study (slope = -1.3149, $p = 0.0311$).

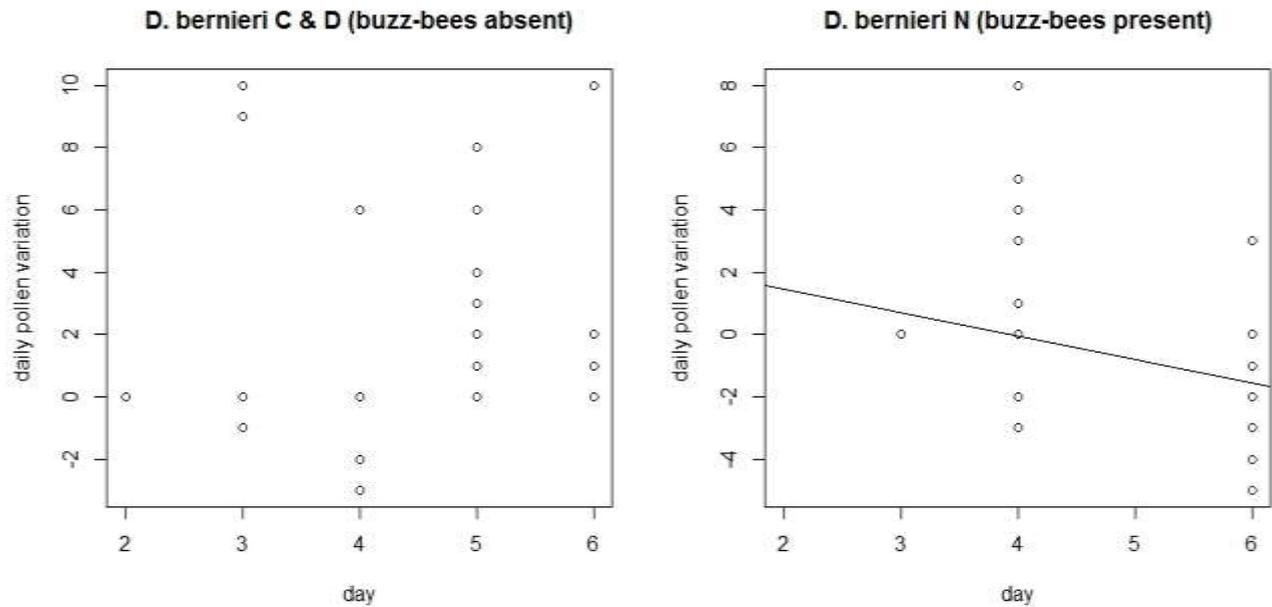


Figure 4. Daily pollen accumulation for *D. bernieri* N and C-D. See text for details.

DISCUSSION

Our first hypothesis is strongly supported by our observations: *D. bernieri*'s bract aperture changed significantly during the day, increasing in the morning and decreasing in the afternoon in accord with the pollinators' activity. Pollinator-related specialisation in *D. bernieri* seems more pronounced than in *D. subternata*, even regarding structures such as the bracts, which are less essentially involved in the pollination process than are the male flowers. *D. subternata*'s bract aperture did not vary during daytime nor night-time: this was anticipated, as for *D. subternata*, which is pollinated by bees as well as by flies and beetles, limiting the access to the flowers at any moment of the day or night could in fact be detrimental.

The first hypothesis being supported by the data, we would expect the second hypothesis (“*D. bernieri* is visited by specific pollinators, while *D. subternata* is visited by a perhaps more abundant, but also more diverse and less specific set of pollinators”) to be verified as well. On the contrary, *D. subternata* vines received very few visits by insects: the most abundant of them, ants, being very unlikely to have any positive role on pollen dispersal. This could be simply due to the fact that our study was carried out too early in respect of the *D. subternata* blooming season, and therefore its potential pollinators were still focused on different, more abundant species. This possibility is supported by the fact that, at the time of our study, the two *D. subternata* vines we surveyed were the only ones we could find with open blossoms. By the end of the study, we could observe numerous *D. subternata* vines carrying many young blossoms. During the same time frame, the *D. bernieri* vines we surveyed received more visitations by pollinators, mainly non-specific *Liotrigona* bees and buzz-pollinating *Nomia viridilimbata*

bees. Our second hypothesis was therefore only partially confirmed: contrary to predictions, *D. subternata*'s structural lack of specialisation was not matched by visits by generalist pollinators; on the other hand, *D. bernieri*'s structural specialisation was matched by the presence of buzz-pollinators as well as abundant, more generalist pollinators.

Our last hypothesis, stating that *D. bernieri* vines visited by buzz-pollinators would gain a larger daily load of pollen than the vines visited only by generalist pollinators, was strongly contradicted by our results. *D. bernieri* vines C and D, visited only by *Liotrigona* bees, did not gain any significant amount of pollen on their female flowers as days passed by. On the other hand, *D. bernieri* vine N showed a significant decline in the pollen load present on female flowers as days passed by, when both *Liotrigona* spp. and *Nomia viridilimbata* bees were present. This trend could be explained if, in presence of efficient buzz-pollinators loading the stigmata with pollen, *Liotrigona* bees switched their behaviour from a 'honest' pollinator to a 'parasitic' one, finding it easier to collect the pollen directly from the stigmata instead of struggling to extract pollen from the enclosed male flowers.

If this were the case, it would fit with the theory proposed by Thompson *et al.* (2002) according to which some insects may have either a positive or negative effect on pollination according to the environmental conditions, acting either as "positive interactivists" (pollinators) or "negative interactivists" (e.g. parasites). Unfortunately this possibility will require further study in Kirindy for confirmation.

In conclusion, it is necessary to point out that the study object of this article has many sources of potential biases. Our sample size is reduced in terms of number of blossoms and plants; the surveys for pollinators' visitations were carried out by different observers on different plants, sometimes belonging to different species, in different times of the day or even on different days. All these are potential, if not certain, sources of pseudoreplication which should not allow us to draw absolute conclusions from our results. However, our results provide baseline information for future studies.

ACKNOWLEDGEMENTS

Many thanks to Scott Armbruster for unveiling pollination ecology to us, and constantly throwing out new ideas for impossible experiments, overwhelming statistics and new studies. We had fun.

REFERENCES

- Armbruster, W.S. (1993) Evolution of Plant Pollination Systems: Hypotheses and Tests with the Neotropical Vine *Dalechampia*. *Evolution* **47**:1480-1505.
- Armbruster, W.S. & Baldwin, B.G. (1998) Switch from specialised to generalised pollination, *Nature* **394**:632.

- Armbruster, W.S. & Baldwin, B. G. (2003) Pollination and evolution of euphorb vines in Madagascar. In Goodman, S. & Benstead, J. (eds.) *The Natural History of Madagascar* Pp. 391-393. University of Chicago Press, Chicago, USA.
- Armbruster, W.S. & Herzig, A.L. (1984) Partitioning and Sharing of Pollinators by Four Sympatric Species of *Dalechampia* (Euphorbiaceae) in Panama. *Annals of the Missouri Botanical Garden* **71**:1-16.
- Armbruster, W.S. & Steiner, K.E. (1992) Pollination ecology of four *Dalechampia* species (Euphorbiaceae) in northern Natal, South Africa. *American Journal of Botany*.
- Armbruster, W.S. *et al.* (2012) Floral Paedomorphy Leads to Secondary Specialisation in Pollination of Malagasy *Dalechampia* (Euphorbiaceae). *Evolution* (in press).
- Thompson, J.N. & Cunningham, B.M. (2002) Geographic structure and dynamics of coevolutionary selection. *Nature* **417**:735-738.