

## SHORT COMMUNICATION

# Pollination ecology and circadian patterns of inflorescence opening of the Madagascan climber *Dalechampia* aff. *bernieri* (Euphorbiaceae)

Marco Plebani<sup>\*,1</sup>, Olive Imanizabayo<sup>†</sup>, Dennis M. Hansen<sup>\*</sup> and W. Scott Armbruster<sup>‡,§,#,¶</sup>

\* Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich

† Karisoke Research Center, Dian Fossey Gorilla Fund International, 4 Ruhengeri, Rwanda

‡ School of Biological Sciences, University of Portsmouth, King Henry Building, King Henry I Street, Portsmouth PO1 2DY, UK

§ Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775, USA

# Department of Biology, NTNU, NO-7491 Trondheim, Norway

¶ Department of Botany, California Academy of Sciences, San Francisco, CA 94118, USA

(Received 21 June 2014; revised 14 October 2014; accepted 15 October 2014)

**Abstract:** Floral morphology often directly influences interactions with pollinators, but less is known about the role of extrafloral structures. We studied the relationship between bract motility, floral structural specialization and pollination in *Dalechampia* aff. *bernieri*, an endemic Madagascan species with floral structures indicating specialized buzz-pollination. We measured circadian bract angles in 47 inflorescences from 11 plants of *D.* aff. *bernieri*; in addition, we recorded any flower-visiting insects observed. The inflorescences had motile bracts with mean angles varying from ~50° at 00h00 to ~90° at 10h45. They were visited by buzz-pollinating *Nomia viridilimbata* bees (Halictidae), but also by non-buzz-pollinating *Liotrigona* bees (Apidae). The temporal pattern of bract motility observed in *D.* aff. *bernieri* may represent an extra-floral specialization to reduce visitation by non-pollinating visitors while maximizing visitation by diurnal buzz-pollinating bees.

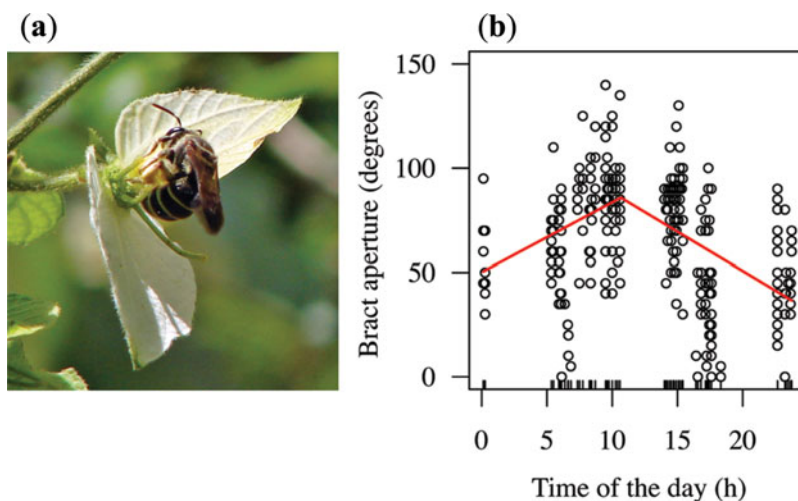
**Key Words:** bract motility, buzz-pollination, *Dalechampia*, *Dalechampia subternata*, floral specialization, *Liotrigona*, Madagascar, *Nomia viridilimbata*, pollination

*Dalechampia* is a pantropical genus of climbers and shrubs comprising over 130 species (Armbruster *et al.* 1993). The plants are monoecious, but with male and female flowers closely adjoined in blossom-like pseudanthia, inflorescences functionally equivalent to a hermaphroditic flower. Two large, showy bracts (modified leaves) subtend the flowers and act like petals to attract pollinators (Armbruster 1993; Pérez-Barrales *et al.* 2013). All 10–12 known species of *Dalechampia* in Madagascar are endemic to the island; they can be divided into two groups based on the openness of their male flowers. Open-flowered species exhibit the structure of male flowers found in nearly all non-Madagascan species of *Dalechampia*, in which the pollen is openly accessible to pollinators. In contrast, closed-flowered species have male flowers in which the sepals remain fused except at their tips (Armbruster *et al.* 2013), making the flowers

functionally equivalent to poricidal anthers found in other plant groups. These structures restrict access to pollen to insects capable of buzz-pollination, the collection of pollen by vibrating ('buzzing') the flowers at high frequencies.

In addition to floral features, extra-floral structures can be important in determining plant-pollinator interactions in *Dalechampia*. Here we focus on the potential role of bract motility, i.e. circadian changes in the angle between bract pairs, in the pollination ecology of *Dalechampia* aff. *bernieri*, endemic of Madagascar. Bracts of most *Dalechampia* species show some degree of bract motility, opening during the day and closing partially or totally at night (Armbruster & Steiner 1992). Bract motility is generally associated with herbivory avoidance (Armbruster & Mziray 1987), but it can also play a role in partitioning pollinators temporally, making the flowers accessible to pollinators only during part of the day (Armbruster & Herzig 1984). In contrast to *Dalechampia* species elsewhere, the relationship between bract motility, floral

<sup>1</sup> Corresponding author. Email: [marcoplebani85@gmail.com](mailto:marcoplebani85@gmail.com)



**Figure 1.** An inflorescence of *Dalechampia* aff. *bernieri* being visited by the buzz-pollinating bee *Nomia viridilimbata* (a). Daily changes in bract aperture observed for *Dalechampia* aff. *bernieri* (b). Solid line: segmented regression fit.

structural specialization and pollination ecology of those found in Madagascar has received little attention.

*Dalechampia* aff. *bernieri* (*Dalechampia* sp. nov. *morondava*, sensu Armbruster *et al.* 1993) is a twining climber with white pseudanthial bracts, a weak floral fragrance and ‘closed’ male flowers (Armbruster *et al.* 2013; Figure 1a). Because its male flowers do not open up fully at anthesis, *D.* aff. *bernieri* is expected to be buzz-pollinated, although buzz-pollination has not yet been observed in *D.* aff. *bernieri* (Armbruster *et al.* 1993).

We expected bract angles of *D.* aff. *bernieri* to change during the day, being wider (bracts more open) when the (predicted) buzz-pollinating bees are more active and bracts being more closed when these pollinators have lower activity, to protect the inflorescence’s flowers from herbivores and/or less efficient pollinators.

The study was conducted at Kirindy Forest (30–60 m asl), a seasonal dry forest approximately 60 km north-east of Morondava, in central-western Madagascar (20.06706°S, 44.65732°E), 5–19 November 2012. Eleven plants of *D.* aff. *bernieri* were surveyed and marked. On each of the plants, 1–12 inflorescences were individually marked, for a total of 47 inflorescences. All plants were within 5 km of each other, no further than 30 m from the side of Conoco road, the main road running through Kirindy forest. For each inflorescence, the angle between the bracts was recorded one to four times each day between 05h00 and midnight, for up to 6 d. The angles between bracts were assessed visually, estimated to the nearest 5°, by the same person (MP) during the whole study. The identity and abundance of insects visiting the inflorescences were assessed by direct observation. Pollinator observation was opportunistically carried out before, during and after measuring the bract angles, for up to 3 h d<sup>-1</sup> per plant. Specimens of the observed flower

visitors were captured, photographed and identified to the lowest possible taxonomic level using Pauly *et al.* (2001).

All statistical analyses were performed using R version 3.0.2. The temporal change in bract angle was analysed with a linear mixed-effect model, with plant identity as a random effect, using function lmer from the package lme4; a second-degree polynomial model was used to assess the non-linear relationship between the bract angle and the time of the day. In case a statistically significant non-linearity was detected, we fitted a segmented regression model to estimate the time of the day at which bract aperture was maximum; this was done using function segmented from the package segmented (Muggeo 2003).

Overall, the bracts of *D.* aff. *bernieri* showed statistically significant non-linear variation in angle with time of day according to the second-degree polynomial model: bract aperture (degrees) = 49.15° + 4.96° h – 0.23° h<sup>2</sup> (SE equals 7.50°, 0.99° and 0.04°, respectively), whereas inflorescences from different individuals showed differences in degree of bract movement. According to the one-break-point segmented regression model, the angle between bracts of *D.* aff. *bernieri* inflorescences was 50.2° (SE = 5.16°) at 00h00 and increased by 3.34° h<sup>-1</sup> (SE = 0.66° h<sup>-1</sup>) toward a peak of ~90° at 10h45, followed by a decrease in bract aperture by 3.75° h<sup>-1</sup> (SE = 0.58° h<sup>-1</sup>) (Figure 1b).

*Dalechampia* aff. *bernieri* was visited by *Nomia viridilimbata* (Halictidae: Nomiinae), which collected pollen by buzzing the flowers, transferring pollen to the stigmas in the process (Figure 1a). This is the first observation of *Nomia* bees pollinating *Dalechampia* flowers and the first documentation of buzz-pollination of *D.* aff. *bernieri*, which confirms the expectation for the species’ pollination strategy based on the structure of its male

flowers. *Dalechampia* aff. *bernieri* was also visited by *Liotrigona* sp. bees (Apidae: Meliponini). These small, dark, stingless bees are incapable of buzz-pollination and, in contrast to reports from previous studies (Armbruster *et al.* 1993), we never observed them contacting the female flowers; these bees were thus acting as pollen thieves rather than pollinators.

Our study is the first attempt at measuring circadian bract movement quantitatively in Madagascan *Dalechampia*. The circadian patterns of bract opening of *D.* aff. *bernieri* match the activity patterns of its observed and expected pollinators, as the temporally restricted diurnal bract opening of *D.* aff. *bernieri* matches the diurnal foraging of specialized *Nomia* bees. Our results strengthen the suggestion that bract movement could be related to specialized floral structures that restrict the access to pollen to a few species of pollinator.

Bract motility might be an element of a pollination syndrome, such that closed-flowered species have motile bracts to favour visitation by diurnal buzz-pollinating insects, while the bracts of open-flowered species always form a wide angle to attract unspecific pollinators at any time of day and night. During our study we could also conduct some observations on *D. subternata*, an open-flowered species known to be pollinated by generalist insects. The bracts of this species show little motility and form a  $\sim 180^\circ$  wide angle around the clock (Armbruster *et al.* 1993; the authors' unpubl. data), thus supporting our hypothesis; yet, this anecdotal speculation will require appropriate testing based on a larger amount of observations and on a comparison among all the Madagascan species of *Dalechampia*.

Future studies should delve deeper into the ecological relevance of bract motility in *D.* aff. *bernieri*. Although the daily changes in bract angles were statistically significant, we did not test for the biological significance of bract motility; bracts that form wide angles may attract pollinators more effectively than bracts at narrower angles by increasing the inflorescence visibility, but the angles observed at night may still be too wide to preclude insect visits to the flowers. This should be addressed experimentally, with more extensive pollinator observations, and by assessing the correlation between individual fitness (i.e. seed production) and the extent of bract motility, which varies markedly between individual plants.

## ACKNOWLEDGEMENTS

We thank: the Tropical Biology Association for financial and logistical support; Dr Brian Fisher for offering us access to the facilities of the Bibikely Biodiversity Center in Antananarivo; Julie Crain of the National Geographic Society archives for retrieving a hard-to-find article; Dr Christopher Philipson and Dr Laura Tamburello for their statistical support and their useful criticisms on early versions of the manuscript.

## LITERATURE CITED

- ARMBRUSTER, W. S. 1993. Evolution of plant pollination systems – hypotheses and tests with the neotropical vine *Dalechampia*. *Evolution* 47:1480–1505.
- ARMBRUSTER, W. S. & HERZIG, A. 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. & MZIRAY, W. 1987. Pollination and herbivore ecology of an African *Dalechampia* (Euphorbiaceae) – comparisons with new-world species. *Biotropica* 19:64–73.
- ARMBRUSTER, W. S. & STEINER, K. 1992. Pollination ecology of four *Dalechampia* species (Euphorbiaceae) in northern Natal, South Africa. *American Journal of Botany* 79:306–313.
- ARMBRUSTER, W. S., EDWARDS, M., HINES, J., MAHUNNAH, R. & MUNYENYEMBE, P. 1993. Evolution and pollination of Madagascan and African *Dalechampia*. *Research and Exploration* 9: 460–474.
- ARMBRUSTER, W. S., LEE, J., EDWARDS, M. E. & BALDWIN, B. G. 2013. Floral pedomorphy leads to secondary specialization in pollination of Madagascar *Dalechampia* (Euphorbiaceae). *Evolution* 67:1196–1203.
- MUGGEO, V. M. R. 2003. Estimating regression models with unknown break-points. *Statistics in Medicine* 22:3055–3071.
- PAULY, A., BROOKS, R. W., NILSSON, L. A., PESENKO, Y. A., EARDLEY, C. D., TERZO, M., GRISWOLD, T., SCHWARZ, M., PATINY, S., MUNZINGER, J. & BARBIER, Y. 2001. Apoidea, Hymenoptera of Madagascar and neighbouring islands. *Koninklijk Museum voor Midden-Afrika Tervuren Belgie Annalen Zoologische Wetenschappen* 286:1–390.
- PÉREZ-BARRALES, R., BOLSTAD, G. H., PELABON, C., HANSEN, T. F. & ARMBRUSTER, W. S. 2013. Pollinators and seed predators generate conflicting selection on *Dalechampia* inflorescences. *Oikos* 10:1411–1428.