Title: Pollination ecology and circadian patterns of inflorescence opening of two species of *Dalechampia* (Euphorbiaceae) in Madagascar

Running title: Pollination ecology of Madagascar *Dalechampia*

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Authors: Marco Plebani[1,2], Olive Imanizabayo[3], Dennis M. Hansen[2], and W. Scott Armbruster[4-7]

1) Corresponding author: marcoplebani85@gmail.com

2) Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich

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

4) School of Biological Sciences, University of Portsmouth, King Henry Building, King Henry I Street, Portsmouth, PO1 2DY

5) Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775 USA

6) Department of Biology, NTNU, NO-7491 Trondheim, Norway

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

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 and floral structural specialization in *Dalechampia* aff. *bernieri* and *D. subternata*, two Madagascan species with floral structures indicating specialized buzz-pollination, and generalized insect pollination, respectively. We measured circadian bract angles in 47 inflorescences from eleven plants of *D.* aff. *bernieri*, and in 12 inflorescences from two *D. subternata* plants. In addition, we recorded any flower-visiting insects observed. *Dalechampia* aff. *bernieri* had motile bracts with mean angles varying from ~50° at 00h00 to ~90° at 10h45. It was visited by buzz-pollinating *Nomia viridilimbata* bees (Halictidae), but also by non-buzz-pollinating *Liotrigona* bees (Apidae). *Dalechampia subternata* bracts were less motile, forming a more or less constant mean angle of ~118° during both day and night; no pollinator visits were recorded. For the specialized *D.* aff. *bernieri*, the temporal pattern of bract motility may represent an extra-floral specialization to reduce visitation by non-pollinating visitors while maximizing visitation by diurnal buzz-pollinating bees.

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 1990, 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 them 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 two *Dalechampia* species endemic to Madagascar, namely *D.* aff. *bernieri* and *D. subternata*. 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 structural specialization, and pollination ecology of those found in Madagascar have 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). *Dalechampia subternata* is also a twining vine, with a strong floral fragrance, and yellow three-lobed pseudanthial bracts; the pollen is presented openly and is easily accessible to pollen-feeding insects (Figure 1b). With these traits we would expect generalized flower visitors; known pollinators include *Euphoria* (Scarabidae: Cetoniinae) and cerambycid (Cerambycidae) beetles, as observed elsewhere in its range (Armbruster ***et al.*** 1993; Armbruster, pers. obs.).

We expected bract angles of the closed-flowered species *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. In contrast, we expected bracts of *D. subternata*, a putative generalist, to remain open throughout day and night, allowing visitation by both diurnal and nocturnal 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* and two

plants of *D. subternata* were surveyed and marked. Ancillary pollinator observations, amounting to ca. 10 hr total, were made on two additional *D. subternata* plants with 4 and 6 open blossoms, respectively, although the blossoms were not marked and the bract angles were not recorded. On each of the main study plants, 1–12 inflorescences were individually marked, for a total of 47 *D.* aff. *bernieri* inflorescences and 12 *D. subternata* 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^{-1} 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 (2014). The temporal change in bract angle was analyzed with a linear mixed-effect model, with plant ID as a random effect, using function *lmer* from package "lme4". For each species, a second-degree polynomial model was used to assess the nonlinear relationship between the bract angle and the time of the day. When a statistically significant nonlinearity was detected at the species-level, 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 package "segmented" (Muggeo 2003).

111 Overall, the bracts of *D.* aff. *bernieri* showed statistically significant non-linear
112 variation in angle with time of day according to the second-degree polynomial model: *bract*
113 *aperture (degrees) = 49.15° + 4.96° h - 0.23° h^2* (SE equals 7.50°, 0.99°, and 0.04°,
114 respectively), whereas inflorescences from different individuals showed differences in
115 degree of bract movement. According to the one-break-point segmented regression model,
116 the angle between bracts of *D.* aff. *bernieri* inflorescences was 50.2° (SE = 5.16°) at 00h00
117 and increased by 3.34° h^{-1} (SE = 0.66° h^{-1}) toward a peak of ~90° at 10h45, followed by a
118 decrease in bract aperture by 3.75° h^{-1} (SE = 0.58 ° h^{-1}) (Figure 2c). In contrast, the angles
119 of the pseudanthial bracts of *D. subternata* did not vary significantly with time of the day
120 (Figure 2d), and the average angle between their bracts was 118° (SE = 4.3°).
121 *Dalechampia* aff. *bernieri* was visited by the *Nomia viridilimbata* (Halictidae:
122 Nomiinae), which collected pollen by buzzing the flowers, transferring pollen to the
123 stigmas in the process (Figure 1a). This is the first observation of *Nomia* bees pollinating
124 *Dalechampia* flowers and the first documentation of buzz-pollination of *D.* aff. *bernieri*,
125 which confirms the expectation for the species' pollination strategy based on the structure
126 of its male flowers. *Dalechampia* aff. *bernieri* was also visited by *Liotrigona* sp. bees
127 (Apidae: Meliponini). However, these small, dark, stingless bees are incapable of buzz-
128 pollination and, in contrast to reports from previous studies (Armbruster ***et al.*** 1993), we
129 never observed them contacting the female flowers. These bees were thus acting as pollen
130 thieves rather than pollinators.
131 Surprisingly, the inflorescences of *D. subternata* were rarely visited; one beetle
132 landed on one inflorescence without touching the flowers, and two ants were observed

apparently eating pollen from open male flowers. This paucity of pollinators was likely due to the very early stage of its blooming season.

Our results strengthens the suggestion that bract movement could be related to specialized floral structures that restrict the access to pollen to a few species of pollinators. Our study is the first attempt at measuring this feature quantitatively in Madagascan *Dalechampia*. The circadian patterns of bract opening of both species match the activity patterns of their observed and/or expected pollinators: the temporally restricted diurnal bract opening of *D.* aff. *bernieri* matches the diurnal foraging of specialized *Nomia* bees; 24-h bract opening in *D. subternata* is consistent with pollination by generalist insects such as cerambycid beetles, which include species active during both day and night (Iwata ***et al.*** 2007).

The results of our study have limitations due to its short duration and small sample size. In particular, our data regarding *D. subternata* are too limited to draw solid conclusions; we presented them as preliminary results, hoping that they may provide a reference for future studies addressing the relationship between floral features, extra-floral structures and pollination ecology in this species.

Future studies should also 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 (see Armbruster and Mziray 1987). Bracts forming wide angles may attract pollinators more effectively than bracts at narrower angles, but the angles observed at night may still be too lareg to preclude insect visits to the flowers. Future studies should address this 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.

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LEGENDS TO FIGURES

Figure 1. The upper panels depict an inflorescence of *Dalechampia* aff. *bernieri* being visited by the buzz-pollinating bee *Nomia viridilimbata* (a), and an inflorescence of *Dalechampia subternata* (b). The lower panels show the daily changes in bract aperture observed for *Dalechampia* aff. *bernieri* (c) and *Dalechampia subternata* (d). Solid line: segmented regression fit. Dotted line: locally averaged line (showing no significant trend).