

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

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4 **Running title:** Pollination ecology of Madagascar *Dalechampia*

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24 **ABSTRACT**

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26 Floral morphology often directly influences interactions with pollinators, but less is known  
27 about the role of extrafloral structures. We studied the relationship between bract motility  
28 and floral structural specialization in *Dalechampia* aff. *bernieri* and *D. subternata*, two  
29 Madagascan species with floral structures indicating specialized buzz-pollination, and  
30 generalized insect pollination, respectively. We measured circadian bract angles in 47  
31 inflorescences from eleven plants of *D. aff. bernieri*, and in 12 inflorescences from two *D.*  
32 *subternata* plants. In addition, we recorded any flower-visiting insects observed.  
33 *Dalechampia* aff. *bernieri* had motile bracts with mean angles varying from  $\sim 50^\circ$  at 00h00  
34 to  $\sim 90^\circ$  at 10h45. It was visited by buzz-pollinating *Nomia viridilimbata* bees (Halictidae),  
35 but also by non-buzz-pollinating *Liotrigona* bees (Apidae). *Dalechampia subternata* bracts  
36 were less motile, forming a more or less constant mean angle of  $\sim 118^\circ$  during both day and  
37 night; no pollinator visits were recorded. For the specialized *D. aff. bernieri*, the temporal  
38 pattern of bract motility may represent an extra-floral specialization to reduce visitation by  
39 non-pollinating visitors while maximizing visitation by diurnal buzz-pollinating bees.

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42 *Dalechampia* is a pantropical genus of climbers and shrubs comprising over 130 species  
43 (Armbruster *et al.* 1993). The plants are monoecious, but with male and female flowers  
44 closely adjoined in blossom-like pseudanthia, inflorescences functionally equivalent to a  
45 hermaphroditic flower. Two large, showy bracts (modified leaves) subtend the flowers and  
46 act like petals to attract pollinators (Armbruster 1990, 1993; Pérez-Barrales *et al.* 2013). All  
47 10–12 known species of *Dalechampia* in Madagascar are endemic to the island; they can be  
48 divided into two groups based on the "openness" of their male flowers. Open-flowered  
49 species exhibit the structure of male flowers found in nearly all non-Madagascan species of  
50 *Dalechampia*, in which the pollen is openly accessible to pollinators. In contrast, closed-  
51 flowered species have male flowers in which the sepals remain fused except at their tips  
52 (Armbruster et al. 2013), making them functionally equivalent to poricidal anthers found in  
53 other plant groups. These structures restrict access to pollen to insects capable of buzz-  
54 pollination, the collection of pollen by vibrating ("buzzing") the flowers at high  
55 frequencies.

56 In addition to floral features, extra-floral structures can be important in determining  
57 plant-pollinator interactions in *Dalechampia*. Here we focus on the potential role of bract  
58 motility, i.e. circadian changes in the angle between bract pairs, in the pollination ecology  
59 of two *Dalechampia* species endemic to Madagascar, namely *D. aff. bernieri* and *D.*  
60 *subternata*. Bracts of most *Dalechampia* species show some degree of bract motility,  
61 opening during the day and closing partially or totally at night (Armbruster & Steiner  
62 1992). Bract motility is generally associated with herbivory avoidance (Armbruster &  
63 Mziray 1987), but it can also play a role in partitioning pollinators temporally, making the  
64 flowers accessible to pollinators only during part of the day (Armbruster & Herzig 1984).

65 In contrast to *Dalechampia* species elsewhere, the relationship between bract motility,  
66 floral structural specialization, and pollination ecology of those found in Madagascar have  
67 received little attention.

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

73 *Dalechampia subternata* is also a twining vine, with a strong floral fragrance, and yellow  
74 three-lobed pseudanthial bracts; the pollen is presented openly and is easily accessible to  
75 pollen-feeding insects (Figure 1b). With these traits we would expect generalized flower  
76 visitors; known pollinators include *Euphoria* (Scarabidae: Cetoniinae) and cerambycid  
77 (Cerambycidae) beetles, as observed elsewhere in its range (Armbruster *et al.* 1993;  
78 Armbruster, pers. obs.).

79 We expected bract angles of the closed-flowered species *D.* aff. *bernieri* to change  
80 during the day, being wider (bracts more open) when the (predicted) buzz-pollinating bees  
81 are more active and bracts being more closed when these pollinators have lower activity, to  
82 protect the inflorescence's flowers from herbivores and/or less efficient pollinators. In  
83 contrast, we expected bracts of *D. subternata*, a putative generalist, to remain open  
84 throughout day and night, allowing visitation by both diurnal and nocturnal pollinators.

85 The study was conducted at Kirindy Forest (30–60 m asl), a seasonal dry forest  
86 approximately 60 km north-east of Morondava, in central-western Madagascar  
87 (20.06706°S, 44.65732°E), 5–19 November 2012. Eleven plants of *D.* aff. *bernieri* and two

88 plants of *D. subternata* were surveyed and marked. Ancillary pollinator observations,  
89 amounting to ca. 10 hr total, were made on two additional *D. subternata* plants with 4 and 6  
90 open blossoms, respectively, although the blossoms were not marked and the bract angles  
91 were not recorded. On each of the main study plants, 1–12 inflorescences were  
92 individually marked, for a total of 47 *D. aff. bernieri* inflorescences and 12 *D. subternata*  
93 inflorescences. All plants were within 5 km of each other, no further than 30 m from the  
94 side of Conoco road, the main road running through Kirindy forest. For each inflorescence,  
95 the angle between the bracts was recorded one to four times each day between 05h00 and  
96 midnight, for up to 6 d. The angles between bracts were assessed visually, estimated to the  
97 nearest 5°, by the same person (MP) during the whole study. The identity and abundance of  
98 insects visiting the inflorescences were assessed by direct observation. Pollinator  
99 observation was opportunistically carried out  
100 before, during, and after measuring the bract angles, for up to 3 h d<sup>-1</sup> per plant. Specimens  
101 of the observed flower visitors were captured, photographed and identified to the lowest  
102 possible taxonomic level using Pauly *et al.* (2001).

103 All statistical analyses were performed using R version 3.0.2 (2014). The temporal  
104 change in bract angle was analyzed with a linear mixed-effect model, with plant ID as a  
105 random effect, using function *lmer* from package "lme4". For each species, a second-degree  
106 polynomial model was used to assess the nonlinear relationship between the bract angle and  
107 the time of the day. When a statistically significant nonlinearity was detected at the species-  
108 level, we fitted a segmented regression model to estimate the time of the day at which bract  
109 aperture was maximum; this was done using function *segmented* from package  
110 "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<sup>2</sup>* (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<sup>-1</sup> (SE = 0.66° h<sup>-1</sup>) toward a peak of ~90° at 10h45, followed by a  
118 decrease in bract aperture by 3.75° h<sup>-1</sup> (SE = 0.58 ° h<sup>-1</sup>) (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

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

135 Our results strengthens the suggestion that bract movement could be related to  
136 specialized floral structures that restrict the access to pollen to a few species of pollinators.  
137 Our study is the first attempt at measuring this feature quantitatively in Madagascan  
138 *Dalechampia*. The circadian patterns of bract opening of both species match the activity  
139 patterns of their observed and/or expected pollinators: the temporally restricted diurnal  
140 bract opening of *D. aff. bernieri* matches the diurnal foraging of specialized *Nomia* bees;  
141 24-h bract opening in *D. subternata* is consistent with pollination by generalist insects such  
142 as cerambycid beetles, which include species active during both day and night (Iwata *et al.*  
143 2007).

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

149 Future studies should also delve deeper into the ecological relevance of bract  
150 motility in *D. aff. bernieri*. Although the daily changes in bract angles were statistically  
151 significant, we did not test for the *biological* significance of bract motility (see Armbruster  
152 and Mziray 1987). Bracts forming wide angles may attract pollinators more effectively than  
153 bracts at narrower angles, but the angles observed at night may still be too large to preclude  
154 insect visits to the flowers. Future studies should address this experimentally, with more  
155 extensive pollinator observations, and by assessing the correlation between individual

156 fitness (i.e. seed production) and the extent of bract motility, which varies markedly  
157 between individual plants.

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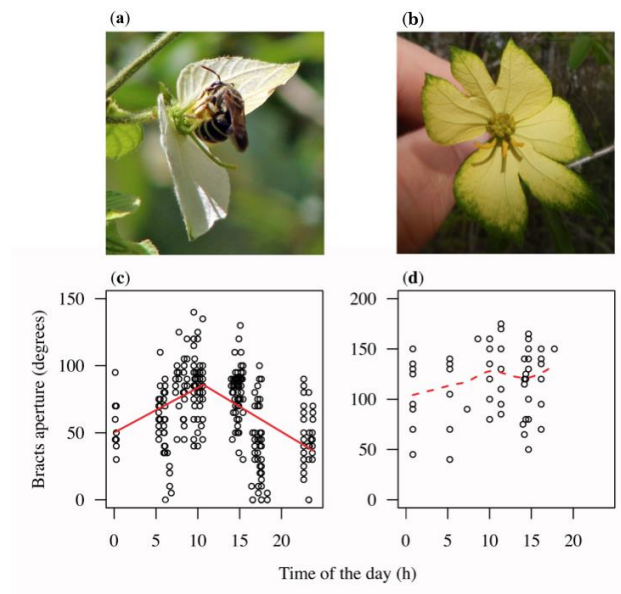
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206 **LEGENDS TO FIGURES**

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

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