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Short communication

"Back-to-bud" strategy in *Nymphaea amazonum* (Nymphaeaceae): A protogynous macrophyte of the Pantanal wetlands

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ABSTRACT

Seven species of *Nymphaea*, a protogynous and night-flowering plant, occur in the Pantanal wetlands. In this study, we report the floral biology of *Nymphaea amazonum* in the Pantanal of Mato Grosso do Sul. We used focal observations and video monitoring throughout the anthesis. Its flowers start to open at dusk. This is followed by an interposing "back-to-bud" stage and finally a full opening from about 04:00 h to 05:00 h. We observed the flowers lasted 3 days and *Cyclocephala* sp. beetles and *Apis mellifera* bees visited them during this time. We compared the floral behavior of *N. amazonum* to Prance's record and found there were several differences. We highlighted the use of non-traditional tools as a complement for a better understanding the floral biology of a previously understudied species.

1. Introduction

Nymphaeceae are an ancient family of flowering plants with worldwide distribution in tropical to temperate regions. It comprises six genera and approximately 60 species of aquatic plants (Schneider and Williamson, 1993). Two genera represent the family in Brazil: *Victoria* and *Nymphaea* with 5 and 18 species recognized, respectively (Sousa and Matias, 2013; Brazil Flora Group, 2015). In the Pantanal wetlands, experts have identified seven species of *Nymphaea* belonging to the subgenera *Hydrocallis*, and all are night-blooming plants (Pott, 1998).

Sexual reproduction in *Nymphaea* is mostly protogynous. Overall, the female and male phases are characterized by floral movements. First, the flower opens 1 day or evening as female, giving access to the stigmatic region, and then closes and reopens as male. Anther dehiscence occurs in second-day flowers, with the stigmatic fluid drying up by this time (Wiersema, 1988).

Aspects of reproductive biology have been documented for many taxa, such the American diurnal species *N. odorata* Aiton (Schneider and Chaney, 1981); *N. elegans* Hooker (Schneider, 1982) and *N. mexicana* Zucc. (Capperino and Schneider, 1985); *N. alba* L. and *N. candida* C. Presl from Holland (Velde, 1986); *N. capensis* Thunb. from Belgium (Orban and Bouharmont, 1995); *N. nouchali* Burm. f. and *N. rubra* Roxb. ex Salisb. from Bangladesh (Begum et al., 2010); and the extinct in the wild *N. thermarum* Eb. Fisch (Povilus et al., 2015). On the other hand, lesser studied species with nocturnal anthesis have been highlighted in the works with *N. blanda* Planch. and *N. rudgeana* G. Mey. from Surinam (Cramer et al., 1975); *N. lotus* L. from the Ivory Coast (Hirthe and Porembski, 2003); and finally *N. pubescens* Willd. from Bangladesh (Begum et al., 2010). The pollination biology of nocturnally flowering species of *Nymphaea* has gone largely unstudied, although experts have already reported on cantharophily (Cramer et al., 1975; Prance, 1980; Wiersema, 1988).

The widespread Neotropical species *Nymphaea amazonum* Mart. & Zucc., which flowers throughout the year, is the most common water lily of Brazil's Pantanal swamps (Pott, 1998). Prance (1980) first reported on the pollination of *N. amazonum* growing in the Pantanal of Mato Grosso. The study described their large, protogynous white flowers which begin opening at dusk and are fully open by sunset (19:00 h). The flowers remain open for only a short amount of time after dawn (between 06:30 h and 07:30 h) and then last for 2 days. *Cyclocephala mollis* beetles are pollinators, and small (unreported species) bees are classified as pollen-gathering visitors.

Since Prance (1980), no one has investigated this species' floral aspects. We therefore engaged in studying the floral biology of *N. amazonum* during fieldwork in the Pantanal of Mato Grosso do Sul, and found some aspects of its floral behavior differed from Prance's original report from the 1980s. While using focal observations and video monitoring throughout the anthesis of the plant, we observed the opening cycle of *N. amazonum*. Consequently, we have new information

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Fig. 1. Floral lifespan of *Nymphaea amazonum* (Nymphaeaceae) found in a Pantanal swamp, Mato Grosso do Sul, Brazil. (a) Flower starts to open at dusk (ca. 18:00 h). (b) Some beetles (*Cyclocephala* sp.) try to enter or leave the flowers during opening. (c) "Back-to-bud" stage where the petals close, but the sepals remain open. (d) First-day flower (female phase) with the stamens and carpellary appendages slightly opened, permitting insect visitors to access the stigmatic region. (e) Second-day flower (male phase) is fully open with the stamens covering the dry stigmatic region. (f) Some *Apis mellifera* bees visit the second-day flowers seeking pollen.

about the floral biology of the *N. amazonum*, as well as its floral visitors and patterns of dichogamy for this night-blooming species. These largely contrast with the pattern previously described.

2. Materials and methods

We carried out our observations during two consecutive nights (13, 14 April 2015) in a population of *N. amazonum* located in a swamp near the Base for Studies of the Pantanal (BEP) (19°34′36.4″ S and 57°01′07.8″ W). The BEP is in the "Passo do Lontra" subregion of Miranda, Corumbá, Mato Grosso do Sul, Brazil. We began our observations at dusk around 17:00 h (all times given in GMT-4) with continuous observations every hour to record flower opening and perform a count of sexual ratio (male or female) in the population. While in the field, we investigated any presence and behavior of pollinators, including *Cyclocephala* sp. beetles.

On the second night, around 17:00 h to 06:00 h, we used an infrared digital video camera attached to a 12 V battery to record the flowering lifespan of some flowers ($N = 3$) over the course of the night, lasting about 13 h. The camera we used is capable of continuous filming, and the images are sharp enough to detect the behavior of flowers from anthesis to wilt.

3. Results and discussion

Nymphaea amazonum flowers start to open at dusk (ca. 18:00 h–19:00 h) with only the sepals and one set of petals widely open (Fig. 1A). During that time, we observed some beetles trying to

enter or exit the flowers, some with pollen coating their bodies (Fig. 1B). Within the 20:00 h to 23:00 h timestamp, the petals closed, but the sepals remained open, giving the impression of a bud stage (Fig. 1C). This "back-to-bud" stage lasted quite long – until 04:00 h. Between 04:00 h to 05:00 h, the flowers opened once again, and all petal sets and stamens fully opened (Fig. 1D–E). At this time, we saw the flower's phases, which depended largely on if the flowers were in the first or second day of their lifespans.

First-day flowers (Fig. 1D) generally did not fully open, and stamens spread to give insect visitors access to the stigmatic region. The tips of the stamens formed a circular wall or palisade around a central pool of stigmatic fluid. On the other hand, in second-day flowers (Fig. 1E–F), the sepals, petals, and most of the stamens fully reflexed. Anther dehiscence occurred in second-day flowers, and stigmatic fluid dried up at or by this time, with staminoids curved over the stigmatic area. From 05:00 h onward, we saw a flurry of visitation by *Apis mellifera* bees seeking pollen (Fig. 1F). Finally, between 05:30 h and 06:30 h, some flowers closed once again (specifically the sepals and petals), while others wilted. The video available in Supplementary Material 1 shows this step-by-step anthesis sequence.

We saw an increase in the ratio of male-to-female flower phases between the two days. On the first day, the ratio was 0.21 (12 male to 55 female flowers), and on the second day, it was 2.65 (69 male to 26 female flowers).

In a protogynous flower like *Nymphaea amazonum*, the male-to-female ratio expectedly increases over the short-term flowering period as all female flowers of the first day become male in the second day. In contrast to our expectations according to the normal behavior of protogynous flowers, we found that the number of male-phase flowers in the second day was higher than that of the first day. However, the video recording allowed us to observe that some male-phase flowers on the second and last day of anthesis wilted, while other second-day flowers closed for a second time. This suggests that *Nymphaea amazonum* flowers lasted for three days. This may explain the higher proportion of male flowers in the second day, as these flowers represent the sum of all female flowers that became male; in other words, these were the first-day flowers that entered into the second day and second-day male-phase flowers that entered into the third day.

In most cases, a flowering cycle of more than 2 days leading to an increase in the percentage of male-phase flowers in a population positively influences bees or syrphid flies' pollination of diurnal *Nymphaea* species (Capperino and Schneider, 1985). A longer flowering cycle might also attract more pollinators by displaying a greater number of open flowers (Wiersema, 1988). This may not be the case in *N. amazonum* because we only saw *A. mellifera* visiting the male-phase flowers, so the combination of twice nightly flower openings and a 2-day male phase may allow this plant to be pollinated in an environment with a lower number of effective pollinators, in this case beetles. Additional study beyond the scope of this note would confirm these suspicions.

In fact, the flowers opening twice nightly in this species may be a mechanism to attract beetles, which are most likely the effective pollinator in this circumstance. Even in the "back-to-bud" phase, the flowers emit a rich fruity odor that attracts these insects. In the population studied, the beetles numbered less than 15 individuals; therefore, the mechanism under discussion could prevent pollen loss once the beetles have forced their way through the petals to reach the reward before the flowers fully opened.

Typically, studying the floral biology of a species requires direct observation of the flower's lifespan. Recent developments in video monitoring – which we have applied during this study – have allowed better sampling of data on insect behavior (see Nakase and Suetsugu, 2016). Here we demonstrate that this technique can helps scientists to follow the entire floral cycle of a species, and especially those with nocturnal anthesis, which can be logistically difficult or inconvenient to keep up with the small nuances of floral behavior. In addition, by using continuous video recording, we could observe small changes in the flowers' behavior that direct observation might not otherwise have yielded. Therefore, the use of non-traditional tools has proven instrumental in gaining more understanding of floral biology, especially in a case like this previously understudied species.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aquabot.2017.04.001.

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