Flora 205 (2010) 818–824

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.de/flora

Night and day service: Distyly and mixed pollination system in *Faramea cyanea* (Rubiaceae)

Pietro Kiyoshi Maruyama[a], Felipe Wanderley Amorim[a,b], Paulo Eugênio Oliveira[a,*]

[a] *Instituto de Biologia, Universidade Federal de Uberlândia - UFU, Cx. Postal 593, CEP 38400-902 Uberlândia, MG, Brazil*
[b] *Departamento de Biologia Vegetal, Universidade Estadual de Campinas - UNICAMP, Cx. Postal 6109, CEP 13083-970 Campinas, SP, Brazil*

ARTICLE INFO

Article history:
Received 26 August 2009
Accepted 3 January 2010

Keywords:
Bee pollination
Cerrado
Faramea hyacinthina
Heterostyly
Moth pollination

ABSTRACT

Currently, pollination is seen as involving more generalist interactions than specialized ones. Supporting this trend, some nocturnal distylous flowers may also receive floral visitors during the day, and since the latter contribute to fruit set, the pollination system is mixed and less specialized. Common among the Rubiaceae, distyly has been regarded as a reproductive strategy which requires a precise and specialized pollination system, and in this important tropical family, environmental disturbance and pollination failure have been used to explain anomalies in distylous features. *Faramea cyanea* Müll. Arg. is a common tree species in forest formations in the increasingly threatened Cerrado biome, the Neotropical savannas in Central Brazil. We evaluated the floral morphology, pollination biology and breeding system of a population of *F. cyanea*. Despite their moth pollination features, flowers were visited by diurnal (bees) as well as nocturnal (moths) pollinators. Experimental results showed that both pollinator groups contributed equally to pollen flow and legitimate pollination. The population presented distyly, isoplethy and heteromorphic self-incompatibility. Although *F. cyanea* did not present exact reciprocal herkogamy between floral morphs, pollination and reproductive success were not impaired. Floral features, which allowed pollination by complementary groups of pollinators, may explain the absence of anomalies in the isoplethy and distylous features in the studied population, anomalies which have been observed in other sympatric distylous Rubiaceae.

Introduction

Distyly is a genetically controlled floral polymorphism, characterized primarily by two floral morphs with reciprocal anther and stigma heights (Barrett, 1992; Barrett et al., 2000; Ganders, 1979). Pin (long-styled) and thrum (short-styled) flowers commonly present reciprocal herkogamy, which functions as a specialized mechanism to promote cross-pollination between individuals of the two floral morphs (Barrett, 1992; Charlesworth and Charlesworth, 1979a; Ganders, 1979; Lloyd and Webb, 1992). Usually, distyly is accompanied by a genetic self-incompatibility system which prevents occurrence of self-fertilization and crossing between individuals of the same morph (Barrett, 1992; Bawa and Beach, 1983; Ganders, 1979). Since distyly is associated with disassortative pollination and pollen deposition on different parts of pollinators' body (Charlesworth and Charlesworth, 1979a; Lloyd and Webb, 1992), reproductive success of distylous species depends markedly on efficient pollination services.

Although heterostyly has evolved independently more than twenty times in the Angiosperms, it is concentrated in insect-pollinated flowers where pollination is especially precise (Endress, 1994). This "precise" pollination requires pollinators to touch specific parts of the flowers in order to transfer pollen and involves, frequently, clear pollination syndromes (*sensu* Faegri and van der Pijl, 1979). These syndromes are sets of floral characteristics that indicate convergent evolution among non-related plant species as adaptation to a pollinator group (Johnson and Steiner, 2000). But currently, pollination systems are mostly seen as involving less specialized interactions (Johnson and Steiner, 2000; Ollerton et al., 2009; Waser et al., 1996), since more generalist systems would be less susceptible to the fluctuation of pollinator availability at a long-term scale (Waser et al., 1996). Nocturnal flowers that last more than one night are interesting examples of more generalized, mixed pollination systems. Some of these flowers receive floral visitors during the day, which contribute to their reproduction (e.g. Muchhala et al., 2009; Wolff et al., 2003; Young, 2002), and day and night pollination shifts constitute an alternative and more effective strategy than single shift specialists (Miyake and Yahara, 1999).

---

[*] Corresponding author.
E-mail addresses: pietrokiyoshi@yahoo.com.br (P.K. Maruyama), amorimfelipe@yahoo.com.br (F.W. Amorim), poliveira@ufu.br, poliveiragm@gmail.com (P.E. Oliveira).

0367-2530/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.flora.2010.01.004

Distyly is one of the most important reproductive strategies in the Rubiaceae, a widespread and predominantly tropical plant family (Bahadur, 1968; Barrett, 1992; Bawa and Beach, 1983; Ganders, 1979; Robbrecht, 1988). Distylous Rubiaceae are well represented in Gallery Forests of the Cerrado biome in Central Brazil, and recent studies have shown deviations from stamen-style reciprocal herkogamy in some populations of otherwise typically distylous species (Coelho and Barbosa, 2003; Consolaro et al., 2005; Consolaro et al., unpubl. data), and these have been associated with environmental changes and pollination failure (Endels et al., 2002; Sakai and Wright, 2008; Sobrevila et al., 1983).

Faramea cyanea Müll. Arg. is a very common tree in forest formations of Cerrado. The genus *Faramea* is reported as typically distylous, but studies focusing specifically on the breeding system and pollination ecology are virtually inexistent for this group. Due to the distyly anomalies reported for other Neotropical Rubiaceae (e.g. Coelho and Barbosa, 2003; Hamilton, 1990), and the precise pollination required for functional distyly, our study aimed to investigate the floral biology and breeding system of *F. cyanea*. In this sense we seek to understand (1) the functioning of distyly and its consequent dependence on precise pollination and (2) the role of its different groups of visitors for the reproductive success of the species.

Materials and methods

Faramea cyanea Müll. Arg. (sub-family Rubioideae, tribe Coussareae) was synonymised with *Faramea hyacinthina* Mart. (Gomes, 2003) but as divergent opinions on the circumscription of this taxon remain, and since there are other names used in literature, we have maintained the name *F. cyanea* in this paper. Fieldwork was carried out from 2003 to 2008 in Panga Ecological Station – PES (19°09′20″S–48°23′20″W; 800 m asl), which belongs to Federal University of Uberlândia, in Uberlândia city, Minas Gerais state. The climate is strongly seasonal, with a warm humid summer (rainy season) and a cold dry winter (dry season). The PES comprises about 400 ha and includes many of the plant formations or physiognomies that characterize the Cerrado biome, varying from grasslands and open savanna to dense forests (Schiavini and Araújo, 1989). The study was conducted in the Gallery Forest associated with the Panga stream.

For phenological studies, nine thrum and 10 pin individuals were marked and accompanied monthly, from September 2003 to September 2004 and from May 2006 to April 2007. Presence of flowers and fruits were evaluated with attribution of intensity scores that varied from zero to three, with zero indicating absence and one, two and three the presence of the phenophase on the intervals 1–25%, 26–75% and 76–100%, respectively (Ribeiro and Castro, 1986). Results were presented as a percentage of the maximum intensity score averaged for each phenophase.

During blooming seasons of 2006 and 2007, we used paths crisscrossing the 40 ha of the studied forest to carry out extensive searches to find blooming *F. cyanea* individuals, which were characterized for floral morph type. We subsequently determined the pin–thrum morph ratio, and deviation in morph ratio from isoplethy was tested using Chi-square test with Yates correction (Zar, 1984).

During the same period, flowers from five individuals of each morph were fixed in 70% alcohol and measured with a calliper (error 0.01 mm) under stereomicroscope. Fifty flowers of each floral morph were collected, 10 flowers per individual. Morphometric data recorded were: corolla tube length; minimal corolla tube diameter; corolla tube opening diameter; anther length; anther height; stigma length; stigma height and stigma–anther separation. Comparison of stigma–anther separation between morphs was used as indicator of presence of reciprocal herkogamy (see Faivre and McDade, 2001). To verify possible dimorphism in pollen grains between pin and thrum flowers, pollen grains from pre-anthesis buds were treated using Erdtman acetolysis (Erdtman, 1960). Acetolysed pollen from five buds from each of the five individuals (the same as above) was pooled on a slide. Five pollen slides per morph were analyzed. Fifty pollen grains per slide were photographed using an optical microscope and the maximum diameter of each grain in polar view was measured using the Image-J software (National Institutes of Health, USA). All morphometric parameters were compared between morphs using Student's *t*-test (Zar, 1984).

Pre-anthesis floral buds in 10 pin and nine thrum individuals were isolated in nylon mesh bags for hand pollination experiments carried out in 2005 and 2006. Hand pollination experiments were done during the day, since flowers were receptive along this period. Pollen donor flowers were also bagged previously. Legitimate cross-pollinations (between individuals of different morphs), illegitimate cross-pollinations (between individuals of the same morph) and self-pollinations were performed for each of the floral morphs. Autonomous apomixis (agamospermy) was tested using previously emasculated flowers which were kept isolated. Fruit development was followed to evaluate pollination treatment success, and a subsample of pistils of each hand treatment ($n = 10$) were collected 24 h after pollination and fixed in a solution of formalin, acetic acid and ethylic alcohol 50% (FAA 50%) for pollen tube growth observation under fluorescence microscopy (Martin, 1959). This procedure allowed us to determine the site of self-incompatibility reaction and pollen tube arresting on the pistils. Flowers of both morphs were also marked in order to estimate natural pollination success.

Floral traits such as flower color, anthesis time and scent production were recorded. Stigmatic receptivity was inferred by the spreading of the stigmatic lobes, a common feature used for Rubiaceae (Coelho and Barbosa, 2003; Consolaro et al., 2005). Nectar production was evaluated, at dawn, using flowers from both morphs (six individuals per morph) previously isolated with nylon mesh bags. Nectar was extracted using disposable glass micro-pipettes and sugar concentration was measured with a hand refractometer (Eclipse®, U.K.). Possible differences in nectar production between morphs were tested with Mann–Whitney *U*-test (Zar, 1984).

In order to determine flower visitors and possible pollinators, diurnal (34 h) and nocturnal (30 h) observations were carried out during 2003 and 2008 blooming seasons, with sessions of 1 h in five different individuals of both floral morphs (three pins and two thrums). During each observation session, we quantified the number of visits by different taxa (large-medium size bees, moths, wasps, butterflies, hummingbirds and small insects) and calculated the visitation frequency for comparison. The difference in the visitation frequencies between the most common diurnal visitors (bees) and nocturnal visitors (moths) was verified using Student's *t*-test (Zar, 1984). Whenever possible, flower visitors were registered and/or captured for identification. In order to determine the role of diurnal vs. nocturnal flower visitors for the pollination of *F. cyanea* we conducted an exclusion experiment during the blooming season of 2008. We bagged flowers at dusk (around 17:00 h) with nylon mesh bags just before opening to exclude nocturnal visitors. Another group of freshly open flowers were marked and only exposed to nocturnal visitors. At dawn (ca. 06:00 h), bagged flowers were released and exposed to diurnal visitors while those previously exposed to nocturnal visitors were bagged. We used for the experiment five individuals for each floral morph, with 8–24 flowers for each pin and 7–24 flowers for each thrum individual. Both the mean number of flowers in different periods (day: 13.60 ± 4.27 flowers; night: 12.30 ± 5.25 flowers; $t = 0.607$, $p = 0.55$) and the mean number of flowers in each floral morph (pin: 11.80 ± 3.25