



FUNCTIONAL DIOECY AND MOTH POLLINATION IN *CABRALEA CANJERANA* SUBSP. *CANJERANA* (MELIACEAE)

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Abstract. Franceschinelli, E. V.; R. M. Carmo, C. M. Silva Neto & J. N. Mesquita Neto. 2015. Functional dioecy and moth pollination in *Cabralea canjerana* subsp. *canjerana* (Meliaceae). *Darwiniana*, nueva serie 3(1): 96-107.

Cabralea canjerana subsp. *canjerana* is a common species in the Atlantic Forest, occurring in several areas of this biome. Yet, little is known about its reproductive strategies. This study aims to understand the reproductive biology of this subspecies, including floral biology, sexual system, and pollination biology. Male and female plants have morphologically similar flowers, but male plants have larger inflorescences with more flowers than female plants. Female flowers remain open and receptive for two days, whereas male flowers last only one day; thus, compensating for the imbalance of the number of flowers per plant between the two sexes. Morphologically similar male and female flowers, as observed in *C. canjerana* subsp. *canjerana*, are rare among dioecious species. The high frequency of moths visiting the flowers, the timing of flower opening (anthesis) and nectar production suggests that moths are the pollinators of this subspecies. In contrast to the floral dimorphism found in *C. canjerana* subsp. *polytricha*, variations in floral morphology are unrelated to the plant sex in subsp. *canjerana*. This and other results found in this study suggest that those two subspecies of *C. canjerana* might be different species.

Keywords. Atlantic Forest; phalaenophily; plant sexual dimorphism.

Resumen. Franceschinelli, E. V.; R. M. Carmo, C. M. Silva Neto & J. N. Mesquita Neto. 2015. Dioecia funcional y polinización por polillas en *Cabralea canjerana* subsp. *canjerana* (Meliaceae). *Darwiniana*, nueva serie 3(1): 96-107.

Cabralea canjerana subsp. *canjerana* es una especie común en la Mata Atlántica del sudeste de Brasil; sin embargo, poco se sabe acerca de sus estrategias reproductivas. Este estudio tiene como objetivo entender la biología reproductiva de esta subespecie, incluyendo su biología floral, sistema sexual y la biología de la polinización. Las plantas masculinas y femeninas tienen flores morfológicamente similares, pero las plantas masculinas tienen inflorescencias más grandes con más flores que las femeninas. Además, las flores femeninas permanecen abiertas y receptivas por dos días, mientras que las flores masculinas duran sólo un día, compensando así el desequilibrio de la cantidad de flores por planta entre los dos sexos. Flores masculinas y femeninas semejantes, como se observan en *C. canjerana* subsp. *canjerana*, son poco comunes entre las especies dioicas. La alta frecuencia de las polillas que visitan las flores, el periodo de apertura de la flor y de la producción de néctar sugieren que las polillas son polinizadores de esta subespecie. Contrastando con el dimorfismo floral encontrado en *C. canjerana* subsp. *polytricha*, las variaciones en la morfología floral no están relacionadas con el sexo de la planta en la subsp. *canjerana*. Este y otros resultados encontrados en este estudio sugieren que estas dos subespecies de *C. canjerana* podrían ser especies distintas.

Palabras clave. Dimorfismo sexual en plantas; falenofilia; Mata Atlántica.

INTRODUCTION

A basic understanding of floral biology is essential for studies related to the pollination of a particular plant species. Flowers have many complex structures adapted to the sexual reproduction. Each part of the flower plays a special role in one or more events occurring during the production, transportation or encounter of gametes. Pollinator behaviour and efficiency are directly associated with the morphology of the flowers that the pollinators visit (Faegri & Van Der Pijl, 1979). Therefore, the morphological, functional, and anatomical characteristics of the flowers are of great importance for understanding the reproductive process of a plant species. These characteristics strongly influence plant-pollinator interaction and its evolution. Furthermore, this interaction plays an important role in gene flow among and within plant populations, shaping deeply their genetic diversity and structure, and consequently affecting their conservation (Rathcke & Jules, 1993; Kearns & Inouye, 1997).

Most angiosperms have hermaphroditic flowers that transmit genes from generation to generation through their female and male function. But, about 6-7 % of angiosperm species are dioecious and have female and male plants (Renner & Ricklefs, 1995). Dioecy in angiosperms may have arisen due to sexual selection, optimising the allocation of resources for reproduction (Bawa, 1980a; Givnish, 1982; Cruden, 1988; Willson, 1994). Furthermore, dioecy is a mechanism that increases the variability and reduces inbreeding depression in populations (Charlesworth & Charlesworth, 1978; Thomson & Barrett, 1981). It has evolved several times during the angiosperm diversification, occurring in many different, no related taxa (Judd et al., 2009) and presenting commonly dimorphism between genders (Eckhart, 1999). Sexual dimorphism in the reproductive traits of dioecious species is well studied (see Eckhart, 1999) and arises either in flower characters, such as size, longevity and morphology (Primack, 1985; Delph et al., 1996; Lenzi & Orth, 2004), nectar production (Bawa & Opler, 1975); or in extra-floral characteristics, such as flower number per inflorescence (Barrett, 1992) and per plant (Delph et al., 2005), inflorescence architecture and size (Rourke, 1989), and others. The occurrence of these

sexual dimorphisms is related mainly to differential sexual requirements that lead to selection on traits that influence male and female reproductive success (Barrett & Hough, 2013).

Hermaphrodite and dioecious species may occur within Meliaceae (Pennington et al., 1981). *Cabralea* is a monospecific genus of this family and *Cabralea canjerana* (Vell.) Mart. includes three subspecies: *C. canjerana* subsp. *polytricha* (Adr. Juss.) Penn., *C. canjerana* subsp. *selloi* (C.DC.) Barreiros and *C. canjerana* subsp. *canjerana* (Vell.) Mart. The sexual system of the genus *Cabralea* is poorly understood (Pennington et al., 1981). A study showed that one of the three subspecies of *C. canjerana*, *C. canjerana* subsp. *polytricha*, is dioecious, has morphological and anatomical differences between male and female flowers and is pollinated by bees (Fuzeto et al., 2001). Recent observations on floral morphology of *Cabralea canjerana* subsp. *polytricha* refute Fuzeto's results, showing similarities between males and female flowers and suggesting moth pollinations (Mesquita Neto et al., unpublished data). Also, dense populations of subspecies *canjerana* in high-montane rainforests of south-eastern Brazil presents male and female plants with similar flowers, and their traits, such as nocturnal anthesis with strong floral sweet odour, suggest moth-pollination. Therefore, an study of morphological and functional characteristics of flowers is essential to identify the sexual system of both taxons. Thus, this paper deals with the floral morphology, biology and the pollination system of *C. canjerana* subsp. *canjerana* with the aim to understand the reproductive biology of this taxonomically complex genus.

MATERIALS AND METHODS

Collection period and study area

The observations and experiments about the floral biology, sexual and pollination system of *C. canjerana* subsp. *canjerana* were conducted during the flowering periods of 2001, 2002, 2003 and 2004. This study was carried out in the Atlantic Forest, in a portion of the Mantiqueira mountain chain (S 22°41'15"; W 45°54'31") located in the municipalities of Camanducaia and Gonçalves in southern Mi-

nas Gerais State, Brazil. The area is environmentally protected. The diversity of ecosystems in the region is high due to the great environmental heterogeneity in the area and variation of altitudes that range from 800 to 2,100m. The climate in the region is temperate humid, with an average annual rainfall of 1,417mm distributed seasonally. The average temperature ranges between 10°C and 22°C throughout the year.

Species studied

Cabralea canjerana subsp. *canjerana* is a tree up to 40m height that occurs in gallery forests and forests of Central and South America. This subspecies is one of the most common plants in the forest fragments of the study area (França & Stehmann, 2004). The voucher of the species studied is *R. B. Torres & C. S. Figueiredo et al. 926* (IAC 38690).

Floral biology and sexual system

Cabralea canjerana plants were observed in several forest fragments and within a Permanent Reserve of about 4000 ha during the flowering periods of 2001. Plants that set fruits were identified initially as female plants, and plants that did not were classified as male. Those plants were marked and their fruit production was verified again in 2002, 2003 and 2004. In addition, to check the sexual system of these plants, their flowers (Table 2) were bagged in pre-anthesis. When these flowers opened, they were hand pollinated. Flowers of female (with fruits) and male (without fruits) plants were hand cross-pollinated with pollen of male plants. Flowers of female plants were cross pollinated with pollen of other female plants. The pollinated flowers of these experiments were re-bagged after being hand-pollinated. Twenty flowers in pre-anthesis of male plants and another twenty flowers in pre-anthesis of female plants were marked and left open to be naturally pollinated. Ten flowers in pre-anthesis of female plants were emasculated marked and bagged to verify the occurrence of agamospermy in this species. The fruit and seed set of different treatments were surveyed. Approximately ten flowers from five plants of each sex were observed daily for four days each year to determine the time and duration of anthesis.

For the anatomical analysis, three flowers of six

male and six female plants were collected. This material was fixed in FAA (mixture of ethanol 70%, formaldehyde 40% aq., glacial acetic acid, 90:5:5 respectively) and subsequently stored in 70% ethanol. Hand sections were performed with the aid of razor blades and styrofoam as support. After cutting, the material was stained with Safranin and astra blue and mounted on microscope slides with glycerol gelatin. The anatomical analysis was performed with an Olympus microscope (BH2 model) and Moticam 350 camera.

In 2002, the pollen of ten flower buds of ten plants of each sex was extracted and mounted on slides. The slides were observed under an optical microscope to obtain direct counts of the number of pollen grains produced per flower. The viability of the pollen grains of flowers of male and female plants was estimated with an indirect method using acetic carmine stain (Dafni, 1992). For this purpose, fresh pollen grains were mounted on slides and observed under an optical microscope. Counts of stained and unstained grains were performed in five fields on each slide. The percentage of stained grains per flower was subsequently calculated from the mean number of stained grains.

To exam the occurrence of sexual dimorphism in the inflorescence, one inflorescence of 10 female and 10 male plants were measured. The main axes and the first secondary axe of those inflorescences were measured in length. The number of flowers was counted in the whole inflorescence and in their first secondary axes as well. Also, the length of ten open flowers from five male and five female plants was measured. To test if morphological differences between genders were statistically significant, one way ANOVA was performed within significance level of 5%. The following morphological differences between male and female plants were tested: the length of flowers, the total number of flowers per inflorescence, the number of flowers on the first inflorescence axe, the length of inflorescence main axis and the length of the first axe.

The duration of male and female flowers were verified to determine functional dimorphism. Thus, the period during which the flowers of female and male plants remain open were observed. In addition, cross-pollinations were performed during the 1st, 2nd, and 3rd days of anthesis in flowers of female plants. On each day, ten flowers from six six

female plants were pollinated. The pollen used in this experiment was collected from the anthers of the flowers of six male plants. The number of flowers that developed into fruits was counted after 42 days.

The amount of nectar produced per flower was measured in ten flowers from six male and six female plants. Due to the small amount of nectar produced per flower, strips of filter paper measuring 25x2 mm were used to collect the nectar and to estimate its volume. Each strip was inserted into the nectar chamber of a flower to absorb the nectar, and the length of the portion of the strip containing the absorbed nectar was measured. This distance was converted into microliter according to a scale constructed by specific distances travelled along similar strips of known volumes of water and sugar solution of equal concentration of *C. canjerana* flower nectar. A pocket refractometer was used to measure the nectar concentration. The nectar was collected with the aid of a microcapillary tube. Due to the small amount of nectar per flower, the nectar from five flowers was combined in the tube prior to the refractometer measurement.

Floral visitors

Floral visitors were observed and captured in male and female flowers between 19:00-5:30h in 2002 and between 18:00-3:00h in 2003. Daytime observations were made from 8:00-18:00h in 2001 and 2002. Pollinator visit frequencies (number of visits in 30min) to *C. canjerana* subsp. *canjerana* flowers were recorded in four different areas. Four randomly selected plants in each area were observed from 20:00 to 22:30h and from 04:00 to 06:30h. Trees located in forest edges were avoided. The observation period lasted 30min per plant and each plant was observed twice or three times per night. Only one inflorescence was observed in each period. A visit was counted as soon as the pollinator visited the flowers to collect nectar. Pollinators visit only few flowers in each visit to an inflorescence of *C. canjerana* (Carmo, 2005). Each time a pollinator visit an inflorescence, we counted only one visit to this inflorescence, independently of the number of flowers visited in it. Each area was sampled for five hours during the flowering peak of *C. canjerana* (October and November).

The occurrence of pollen grains of *C. canjerana* on the bodies of the visitors collected was verified with small pieces of glycerinate gelatine, which were rubbed near the mouthparts and legs of the moths. The gelatine pieces were subsequently mounted on slides that were observed with an optical microscope.

RESULTS

Floral morphology and anatomy

Flowers of male and female plants are morphologically very similar and have the following characteristics: five free sepals and petals, whitish petals, absence of nectar guides and ten stamens with filaments completely fused forming the staminal tube (Fig 1). The anthers are located on the inner face of this tube and show longitudinal introrse dehiscence. The ovary is semi-inferior with five carpels and five loci, each comprising two superposed ovules (Fig. 2). Flowers of female and male plants have a developed gynoecium. No abnormalities were found in the ovary of the male flowers. Anatomically, these structures are identical in the two floral types. The stigma is central and presents an expanded epidermis consisting of elongated cells forming a dense coat of papillae. The stigma mesophyll includes a large number of secretory cells. In the style, the transmitting tissue is formed of long epidermal cells surrounding a central cavity. The ovule is anatropous, presenting micropyle, funicle, inner and outer integument and nucellus in both floral types. But, the embryo sac could not be visualised precisely either in male or female flowers. The anthers of flowers of both sexes are morphologically similar. However, viable pollen grains are produced in greater amounts in flowers of male plants (see below, Floral biology and sexual system). The viable pollen grains have the following average dimensions: 31.5 μm (± 0.8) in the equatorial axis and 33.0 μm (± 0.8) in the polar axis.

Male and female flowers have a developed nectary disc at the base of the flower between the stamen tube and the pistil (Fig 2). The inner surface of this disc is coated with large number of trichomes. The mesophyll of this disc has several glandular cells that do not differ in number or in size between the

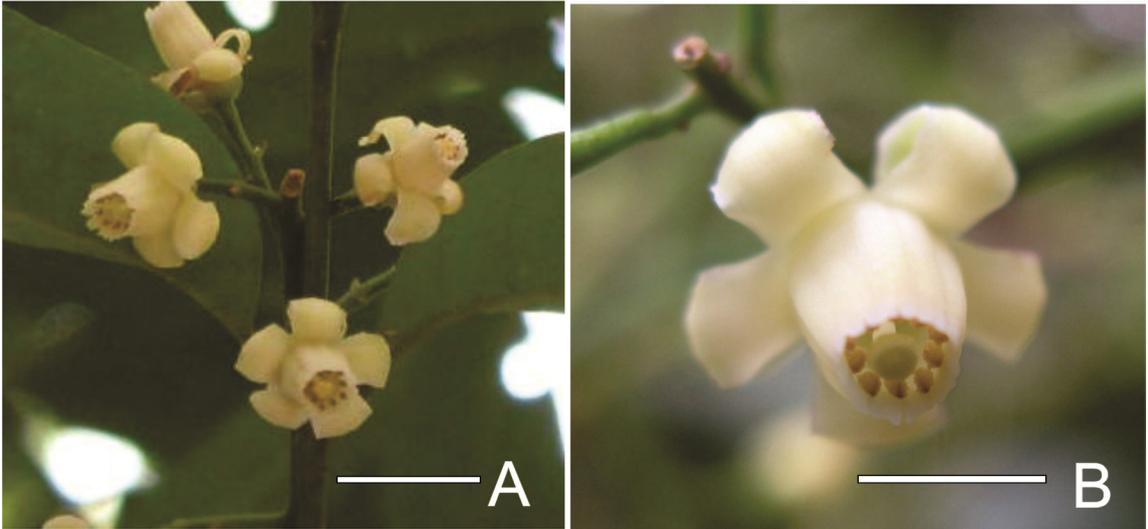


Fig. 1. *Cabralea canjerana* subsp. *canjerana*. **A**, female flower. **B**, male flower. Scales: A = 10 mm; B = 5 mm. Color version at <http://www.ojs.darwin.edu.ar/index.php/darwiniana/article/view/599/646>.

two floral types. The disc has no vascular bundles; however, bundles of floral receptacle reach its base.

There is no noticeable difference in flower size between male and female plants. Flowers of male (9.51 ± 0.54 mm) and female (9.31 ± 0.54 mm) plants have similar length ($t = 1.024$; DF: 14; $p = 0.323$). Likewise the morphology of the staminal tubes, which may be straight or curved, is not related to the sex of the flower, and both forms of tube may occur in the same plant, either male or female. Nevertheless, inflorescences of male plants have larger secondary axes and greater number of flowers than female plants (Table 1, Figs. 3 and 4). The male plants presented inflorescences with secondary axes up to seven times longer than female plants (Fig. 4) ($F(4,17) = 34.04$; $p = 0.000$). Male and female plants presented inflorescences with similar main axe length, but the inflorescence of male plants set on average 110 more flowers than female ($p = 0.000$). The secondary axes of male plant inflorescences set up to 19 times more flowers than the ones of female plants ($p = 0.000$) (Fig. 4).

Floral biology and sexual system

Plants of *C. canjerana* subsp. *canjerana* bloom from September to November, with a flowering

peak in October. Anthesis begins at approximately 17:30h. The anthers open about 40 min later than the petals. The flowers release a strong, sweet fragrance at night. Each male flower produces, on average, 180 (± 91.56) grains per anther. These pollen grains are 90% viable. Each female flower produces, on average, only 10 (± 9.89) grains per anther, being mostly unviable (92%). Each female flower produces 10 ovules. Thus, the pollen/ovule ratio (P/O) is approximately 180. On the first day after anthesis, female flower produces, on average, 11 μ l (± 1.32) of nectar, with an average sugar concentration of 12% (± 0.6). Male flowers produce similar volume (10 μ l, ± 1.02) and nectar concentration (13%, ± 0.4). We visually detected the presence of nectar in flowers that had remained open for two days. However, nectar analysis was only done in first-day flowers.

The observed plants did not change sex during the 4 years of study. Plants that produced fruits did not presented flowers with anthers full of viable pollen at all. Plants that produced flowers with viable pollen did not set fruit during the four-year study. Flowers of female plants remained open for up to three days. The pollinated first- and second-day flowers produced fruit (90% and 75% fruiting, respectively). The flowers pollinated on

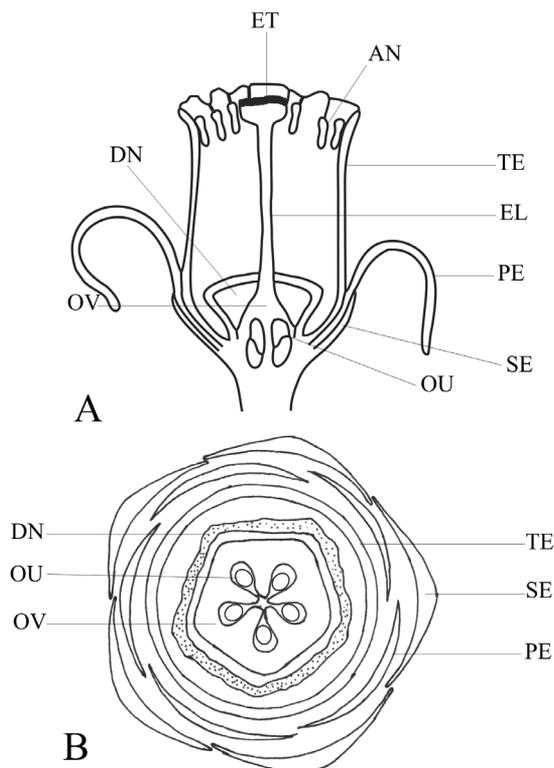


Fig. 2. *Cabralea canjerana* subsp. *canjerana*. **A**, diagram of a longitudinal section of a female flower. **B**, female flower, floral diagram (anthers not represented). Abbreviations: DN, nectary disc; OU, ovule; OV, ovary; ST, stem tube; SE, sepal; PE, petal; SL, stylus; SG, stigma; AN, anther.

the third opening day did not produce fruit. Six of them fell from the plant during hand pollination. Flowers of male plants last only one day. Manually pollinated flowers of male plants did not set fruit at all (Table 2). No flowers of male plants produced fruit. Manually pollinated female flowers produced fruit only if they received pollen from male flowers. The number of seeds per fruit resulting from this treatment ranged from 7 to 10. The female flowers did not produce fruit by agamospermy. The fruits that set naturally (control) produced 1-10 seeds per fruit.

The fruits of this subspecies are red, loculicidal capsules with a fleshy pericarp, bitter and astringent. They contain 1-5 diaspores, which consist of one or two seeds united by the aril. The seeds are ellipsoids and are partially (sometimes completely)

covered by the aril. The dehiscence of the fruits occurs primarily before the fruits fall. This event results in the exposure of the seeds. In certain cases, closed ripe fruit, open fruits and flower buds occur simultaneously on the same plant due to the long period of fruit development.

Floral visitors

The flowers of *C. canjerana* subsp. *canjerana* were visited by moths for nectar (Fig. 3B). The visits began at approximately 17:30h and continue until approximately 5:30h, with a peak in the number of visits between 20:00-22:00h. Moths belonging to five families were observed visiting the flowers: Noctuidae (represented by nine morphospecies), Geometridae (five morphospecies); Arctiidae, Pyralidae, and Pyromorphidae (one morphospecies each). Three morphospecies belonging to Noctuidae, two Geometridae and one Pyromorphidae were the most frequent visitors.

The frequency of visits to an inflorescence in the beginning of the night (20:00 – 22:00h) was $8.81 (\pm 3.94)$ visits per half an hour of observation. In the end of the night (04:00-06:00hs), the visit frequency was lower (3.53 ± 3.85 visits per half an hour of observation). Each visit lasted from 3 to 47 seconds. The behaviour of visitors to the two types of flowers did not differ. Moths frontally approached the inflorescences of both the male and female plants and landed on one of the flowers to collect nectar. After visiting a particular flower, the moth visited another flower of the same inflorescence in 37% of the observed cases ($n=35$). In these visits, the moths contacted the reproductive structures of the flower with their mouthparts. Pollen grains of *C. canjerana* subsp. *canjerana* were found on all moths examined ($n=36$) of all taxa mentioned. Counting the grains on the moth bodies was not possible because large numbers of moth scales adhered to the glycerinate gelatine when it was rubbed against the body of the moth to collect pollen grains.

In 2003, we observed the exotic bee *Apis mellifera* (Hymenoptera, Apidae) visiting flowers of *C. canjerana* subsp. *canjerana* for a short period of time. These visits occurred at approximately 17:45h and ended before nightfall. The frequency of visits by this species was five bees per plant per



Fig. 3. *C. canjerana* subsp. *canjerana*. A, female inflorescence. B, male inflorescence, a moth is landed on a male flower. Color version at <http://www.ojs.darwin.edu.ar/index.php/darwiniana/article/view/599/646>.

hour. *Apis mellifera* visits flowers of few plants in the area and they tend do not move from one plant to another. During these visits, the bees approached the inflorescences frontally and continued flying for a few seconds before landing on a flower to collect nectar. *Apis mellifera* visited only flowers that were recently opened.

DISCUSSION

The dioecy of *C. canjerana* subsp. *canjerana* is confirmed by the finding that the flowers of male plants do not produce fruits during the four observed years, even if they are manually pollinated. Indeed, the male flowers have a well-developed gynoecium with well-formed ovaries and ovules possessing normal integuments and a nucellus, although we could not confirm a normal embryo

sac. Moreover, the female flowers have anthers although they produce few pollen grains with low viability. Here, the dioecy is mainly functional based on anthesis duration, pollen amount and viability, and on flower number per inflorescence.

Delph et al. (1996) observed differences between the size of the male and female flowers in approximately 85% of 436 analysed dioecious species. However, close morphological similarities between male and female flowers have previously been observed in several other dioecious species belonging to different families (Lloyd, 1980; Humeau et al., 1999; Fuzeto et al., 2001; Li et al., 2010). But, male and female flowers which are morphologically and anatomically identical, especially in the characteristics of the ovary and ovules, as occurs in *C. canjerana* subsp. *canjerana*, is a rare condition in nature. According to Freeman et al. (1997), natural selection tends to limit floral di-

Tabla 1. Morphometric comparison (average and standard deviation) between male and female inflorescence. Abbreviations: ns, non-significant statistical difference at 5% level; **, significant statistical difference at 1% level.

Plant sex	Inflorescence main axe lenght (cm) ^{ns}	Inflorescence 1° secondary axe lenght (cm)**	N° of flowers per inflorescence**	N° de flowers in the 1° secondary axe**
Male	15,1±3,6	10,3±2,0	138,1±16,90	24,1±4,67
Female	14,6±3,4	2,7±1,0	25,9±11,89	5,1±1,72

morphism to ensure successful pollination because pollinators must visit flowers of both sexes indiscriminately.

These similarities were not observed in species of *Trichilia*, another genus of Meliaceae family. In these species, male flower presented residual ovary and deformed ovules (Souza et al., 2001). In the subspecies *polytricha* of *C. canjerana*, the ovules of the male flowers are small and morphologically deformed, male and female flowers have different size and staminal tube shape (Fuzeto et al., 2001). The corresponding differences were not observed in the *canjerana* subspecies. Here, the variations in these characteristics have no relation with the sex of the plant because flowers of different sizes and with curved or straight staminal tubes occur on the same plant. *C. canjerana* subsp. *canjerana* presents sexual dimorphism in some inflorescence characteristics, such as larger male inflorescences with higher number of flowers than female inflorescences.

In dioecious species, the relationship between the amount of pollen produced per flower and the number of ovules (pollen/ovule ratio - P/O) tends to be high. In *C. canjerana* subsp. *canjerana*, P/O is low. According to Cruden's prediction (Cruden, 1977), low P/O values are common among autogamous species. However, the low pollen production per flower in this subspecies may be balanced by the higher production of male flowers per inflorescence and plant, which increases the amount of pollen grains in the system. In fact, tropical dioecious trees tend to produce large amounts of pollen per plant (Opler & Bawa, 1978) to cover possible losses (Cruden, 1977). Additionally, the female flowers last up to two days; although the stigmas are more receptive on the first day of flower opening, the maintenance of flower viability for more than one day may be advantageous for the

plant. Besides, it is energetically less expensive to keep unfertilised flowers open for more than one day rather than to produce fresh flowers every day (Bezerra & Machado, 2003). The difference on flowers duration between male and female plants can be considered a sexually dimorphic character as well.

Although the nectaries of *C. canjerana* subsp. *canjerana* are well developed, they produce a low volume of nectar with a low concentration of sugar. These characteristics can be related to the lack of vascularity of the nectary tissue. The amount and concentration of sugar in nectar is directly related to the type and behaviour of the pollinator guild that consume the nectar (Heinrich & Raven, 1972; Kearns & Inouye, 1993). Species pollinated by moths generally produce nectar with a low sugar concentration in small amounts, as is the case in *C. canjerana* subsp. *canjerana* (Willmott & Burquez, 1996; Groman & Pellmyr, 1999). In this species, nectar is the single reward offered to pollinators by both the male and the female flowers. According to Bawa (1980b), visitors respond to differences in the quantity and quality of floral resources within a population. If male and female flowers produced different amounts of nectar or nectar with different concentrations of sugar, the rates of visits to male and female flowers might differ between sexes and interfere in the success of the pollination process. The great morphological and anatomical likeness between the nectaries of the female and male flowers explains the similarity in the sugar concentration and the amount of nectar produced in these two types of flowers.

The flower features of *C. canjerana* subsp. *canjerana*, including nocturnal anthesis, a whitish colour, the absence of nectar guides, a strong sweet scent, and a low production of nectar with a low

sugar concentration are consistent with the phalaenophily syndrome (Faegri & Van Der Pijl, 1979). Moreover, the high frequency of moths visiting flowers suggests the importance of these insects as pollinators of this subspecies. The size and morphology of the pollen grains are also related to the phalaenophily syndrome. Moths and butterflies, like birds, tend to carry larger pollen grains than those carried by bees (Harder, 1998).

Although the flowers of *C. canjerana* subsp. *canjerana* produce small amounts of nectar with a low sugar concentration, this nutritional level may be sufficient for moths, which have a low metabolism (compared to hawkmoths, for example) and

can survive on small quantities of sugar. The production of small amounts of nectar can also favour the reproductive success of subsp. *canjerana* because moths may need to visit several flowers to collect enough sugar to meet their metabolic needs. Multiple visits to the same plant of a dioecious species can increase the pollen load that has to be transported to other plants sometimes a long distance away. Moths can carry pollen grains for long periods of time and over long distances between consecutive visits to flowers (Young, 2002). It is probable that this characteristic contributes to gene flow among male and female plants of subsp. *canjerana*. Moths do not take a large percentage

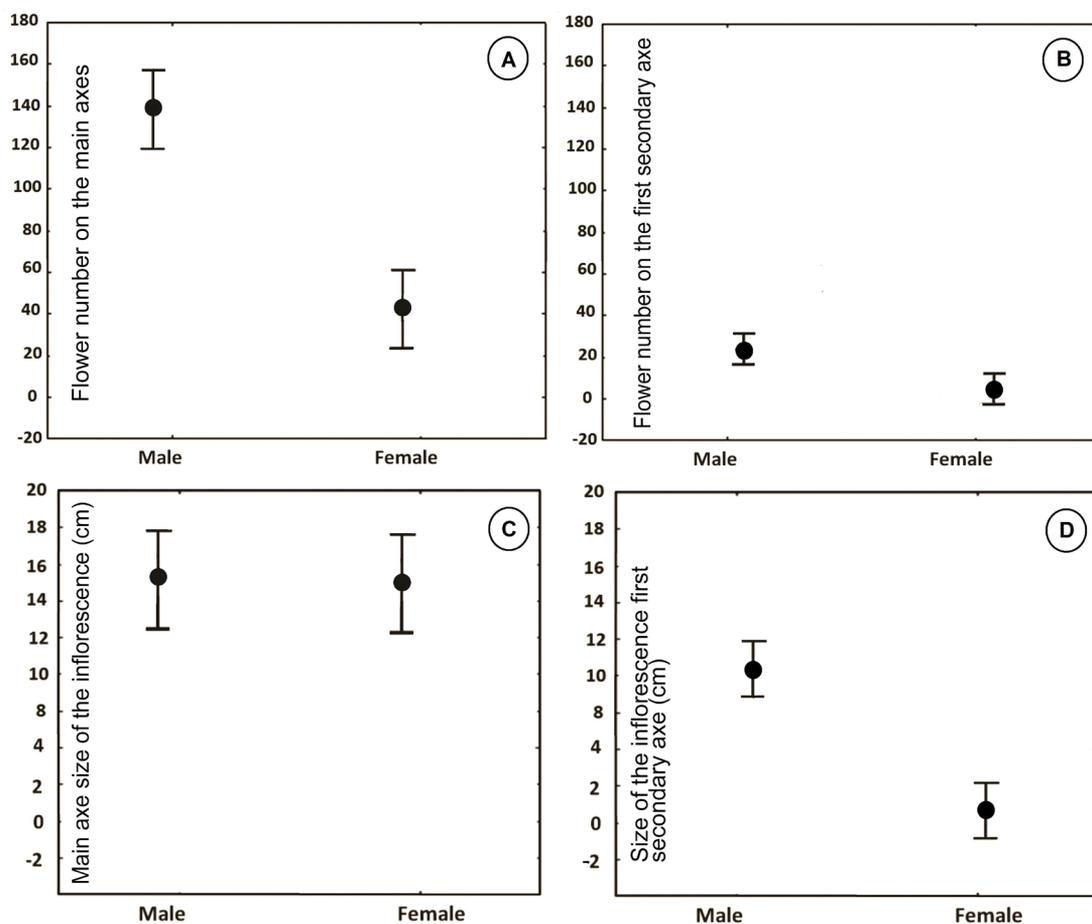


Fig. 4. Graphs showing differences between flowers abundance (A and B) and male and female inflorescences sizes (C and D)

Tabla 2. Pollination treatments performed on flowers of *C. canjerana* subsp. *canjerana*.

Treatments	Flowers	Number of fruits	Mean number of seeds/fruits± SD
Female x male	25	21	7,47 (±1,26)
Female x female	21	0	-
Male x male	19	0	-
Agamospermy	10	0	-
Control female	20	15	5,58 (±0,83)
Control male	20	0	-

of the pollen produced in a single visit (Young, 2002). This observation may explain the low pollen production per flower of this subspecies.

Fuzeto et al. (2001) found that *C. canjerana* subsp. *polytricha* has diurnal anthesis and is pollinated by bees. The visits of *A. mellifera* bees to flowers of *C. canjerana* subsp. *canjerana* most likely do not result in pollination because the anthers generally remain closed during the day and the frequency of visits to the flowers by these bees is very low. If *C. canjerana* subsp. *polytricha* is bee-pollinated as proposed by Fuzeto et al. (2001), the contrasting pollination syndrome could represent an interesting mechanism of reproductive isolation between the two subspecies because the pollinators of one would not visit the flowers of the other; besides they do not co-occur (subsp. *polytricha* occurs in cerrado and subsp. *canjerana* in forest habitats) so pollen exchange between them is practically impossible. Differences in pollination system furnish an indication that these two taxa may be considered two different species. However, we have observed that flowers of this subspecies open during the night in cerrado areas of Minas Gerais and Goiás States in Brazil. Then, more detailed observations are important to check the real pollination system of subspecies *polytricha*.

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