Alexandre Silva, Celice; Faria Vieira, Milene
FLOWERING AND POLLINATORS OF THREE DISTYLOUS SPECIES OF Psychotria (Rubiaceae) CO-OCCURRING IN THE BRAZILIAN ATLANTIC FOREST
Revista Árvore, vol. 39, núm. 5, septiembre-octubre, 2015, pp. 779-789
Universidade Federal de Viçosa
Viçosa, Brasil

Available in: http://www.redalyc.org/articulo.oa?id=48842815001
FLOWERING AND POLLINATORS OF THREE DISTYLOUS SPECIES OF Psychotria (Rubiaceae) CO-OCCURRING IN THE BRAZILIAN ATLANTIC FOREST

Celice Alexandre Silva and Milene Faria Vieira

ABSTRACT – This study investigates the flowering and pollinators of the floral morphs of three co-occurring distylous species, Psychotria conjugens Müll., P. hastisepala Müll. Arg., and P. sessilis Vell., in two consecutive flowering seasons in an Atlantic Forest fragment in southeastern Brazil. The species have diurnal, cream-colored, tubular, nectariferous flowers and their flowering occurs in the rainy season, from September to April, with little or no overlapping between species, characterizing a staggered flowering. The flowering of the long- and short-styled floral morphs of each species was synchronous, but the number of open flowers per day per morph tended to vary in each flowering season. These numbers were higher in P. sessilis and P. conjugens and, probably, resulted in higher total numbers of visits on its flowers (up to 1084 visits in P. sessilis and 756 in P. conjugens), compared to that observed in P. hastisepala (up to 71). There was a higher frequency of visits to long-styled flowers of all species. The bee Ariphanarthra palpalis was a common pollinator to all species. This bee is native to Brazil, solitary, considered relatively rare and its host plants were unknown. Other native bees (Melipona spp.) also visited the flowers of the Psychotria species. The availability of flowers with similar floral features over eight months, the staggered flowering and common pollinators appear to be part of a strategy to attract floral visitors, minimizing the competition for pollinators and then favoring the legitimate pollination of these plants.

Keywords: Bees; Sequential flowering; Frequency of visits.

FLORAÇÃO E POLINIZAÇÃO DE TRÊS ESPÉCIES DISTÍLICAS DE Psychotria (Rubiaceae) COOCORRENTES NA MATA ATLÂNTICA BRASILEIRA

RESUMO – Este estudo objetivou investigar a floração e polinização dos morfos florais de Psychotria conjugens Müll., P. hastisepala Müll. Arg. e P. sessilis Vell., espécies distílicas e coocorrentes. As observações foram feitas em duas florações consecutivas, em um fragmento de Mata Atlântica do Sudeste do Brasil. As espécies têm flores com antese diurna, cor creme, tubular, nectaríferas. A floração ocorre na estação chuvosa, de setembro a abril, com pouca ou nenhuma sobreposição entre as espécies, caracterizando uma floração escalonada. A floração dos morfos florais, longistilo e brevistilo, de cada espécie foi síncrona, mas o número de flores abertas por dia por morfo teve a variar em cada floração. Esses números foram maiores em P. sessilis e P. conjugens e, provavelmente, resultaram em maiores números totais de visitas nas suas flores (até 1.084 visitas em P. sessilis e 756 em P. conjugens), em comparação com o observado em P. hastisepala (até 71). A frequência de visitas foi maior nas flores longistilas de todas as espécies. A abelha Ariphanarthra palpalis foi o polinizador comum a todas as espécies. Esta abelha é nativa do Brasil, solitária, considerada relativamente rara, e suas plantas hospedeiras eram desconhecidas. Outras abelhas nativas (Melipona spp.) também visitaram as flores das espécies de Psychotria. A disponibilidade de flores com características florais semelhantes ao...
longo de oito meses, a floração escalonada e polinizadores em comum parecem ser partes de uma estratégia para atrair visitantes florais, minimizando a competição por polinizadores e, consequentemente, favorecendo a polinização legítima dessas plantas.

Palavras-chave: Abelhas; Floração sequencial; Frequência de visitas.

1. INTRODUCTION

The key event in the reproductive biology of angiosperms is flowering (PRIMACK, 1985) and studies on this phenophase are fundamental to understand their regulatory factors (MARTIN-GAJARDO; MORELLATO, 2003). In tropical species, the onset of flowering is usually triggered by the greater rainfall intensity (rainy season) favoring synchronous flowering within populations (PENHALBER; MANTOVANI, 1997). In a population, the number of simultaneously open flowers varies between plant individuals of the same species and the amount of floral resources influences the pattern of pollinator visits (KARRON; MITCHELL, 2012).

The flowering of distylous species of Psychotria, analogous to the above description for tropical species, is mostly synchronous, annual and occurs during the rainy season (CASTRO; OLIVEIRA, 2002; COELHO; BARBOSA, 2004). This phenological pattern has been ascribed to the attraction of pollinators, in particular of bees and butterflies, and to obtain greater reproductive success of each floral morph (CASTRO; OLIVEIRA, 2002), which in turn is related to the amount of flowers available, visitation frequency and pollinator behavior (DONALDSON et al., 2002; SAKAI; WRIGHT, 2008).

Interestingly, the co-occurring distylous Psychotria species tend to have sequential flowering (ALMEIDA; ALVES 2000; CASTRO; OLIVEIRA, 2002; PEREIRA et al., 2006) and common pollinators (CASTRO; OLIVEIRA, 2002), but no evidence of interspecific competition have been found (ALMEIDA; ALVES, 2000; CASTRO; OLIVEIRA, 2002).

The present study aims to investigate the flowering (inflorescences per plant, flowers per inflorescence and open flowers per plant per day and number of flowers per peak of flowering) and the identity of the pollinators of each floral morphs of Psychotria conjugens Müll. Arg., P. hastisepala Müll. Arg. and P. sessilis Vell. Answers to the following questions were sought: (a) Is the flowering of the floral morphs of each species synchronous? (b) Is the flowering of the different species synchronous? (c) Which are the pollinators? (d) Do the species have pollinators in common? (e) Are the visitation frequencies of the pollinators to the floral morphs of each species similar?

2. MATERIAL AND METHODS

2.1. Study area and species

The study was conducted at the Estação de Pesquisa Treinamento e Educação Ambiental Mata do Paraíso (EPTEAMP), in the municipality of Viçosa (20° 45’S, 42° 51’W), southeastern Brazil, at about 650 masl. The climate of Viçosa is characterized by an average annual temperature of 19 °C and a mean annual rainfall of 1300-1400 mm, most of which falls between September and March, and a relative humidity of 80-85%. The original vegetation of the study area was part of the Atlantic forest classified by VELOSO et al., (1991) as submontane semideciduous forest. The EPTEAMP is an environmentally protected secondary forest, covering an area of about 194 ha.

Psychotria conjugens and P. hastisepala are subshrubs (plant height 1.0 - 2.5 m), with distribution restricted to Brazil (ANDERSSON, 1992), in phytogeographical domains of savanna and Atlantic Forest (TAYLOR et al., 2015). P. sessilis is a shrub (plant height 1.5 - 3.5 m) also found in several other South American countries (ANDERSSON, 1992). The studied species grow in the understory of EPTEAMP and are distylous (PEREIRA et al., 2006). The flowers of the three species are diurnal, nectariferous, tubular, cream-colored and last one day (PEREIRA et al., 2006).

The fruits are drupaceous, blue, or dark purple at maturity and ornithochorous. Each fruit produces 1-2 seeds (pers.comm.). Voucher specimens were deposited in the VIC (26,963, 26,964 and 26,974).
2.2. Flower production

Two consecutive flowering seasons (September 2004 to April 2005 and September 2005 to April 2006) were monitored weekly. In the first flowering season, 19 plants with short-styled flowers (S) and 23 long-styled flowers (L) of *P. sessilis* were labeled, 09 S and 04 L of *P. conjugens* and 22 S and 18 L of *P. hastisepala*. In the second flowering season, 10 S and 10 L plants of each species were labeled. Of each plant and flowering season, the numbers of inflorescences/plant and open flowers/plant/day were recorded, except for *P. sessilis*. From this species, in view of its height and the high number of inflorescences and flowers, these data were recorded weekly on three randomly chosen branches per year and plant. The number of flowers/inflorescence was recorded only for the first flowering season (N = 30 for each species and morph), in all species.

2.3. Flower visitors

The flower visitors were observed, captured and killed, and later mounted on entomological pins, labeled and identified with the help of a specialist. Voucher specimens were deposited in the Museu Regional de Entomologia da Universidade Federal de Viçosa (UFVB).

At the flowering peak of each species in 2004/2005 and 2005/2006, the visitation frequency of flowers of both morphs was recorded in plants at least 30 meters away from the forest edge, to minimize the edge effects. For this measurement, in both flowering seasons, from 7:00 to 15:00, on three consecutive days for *P. sessilis* and four consecutive days for *P. hastisepala*, flower visitors were identified and their visits counted. The visits were counted in eight blocks of 30 min, alternating the floral morphs, resulting in a total of 12 and 16 h of observation/flowering season for *P. sessilis* and *P. hastisepala*, respectively. The flower visitors of *P. conjugens* were only identified and quantified in the flowering of 2005/2006, similarly as described for *P. sessilis* (12 h of observation).

Flower visitors were considered legitimate pollinators if they touched anthers and stigmas and collected pollen or nectar (legitimate visits).

2.4. Statistical analyses

Statistical analyses were conducted using the program “General Linear Model,” of Statistica version 5.5 (STAT SOFT, 2002). In the analysis of variance, the One - way ANOVA test was applied (ZAR, 1999).

3. RESULTS

3.1. Flowering

In both monitored flowering seasons, *Psychotria sessilis* (Fig. 1AB) flowered first, from September to December 2004 (Fig. 1A) and from September to November 2005 (Fig. 1B), in the rainy season. In these periods, synchrony of the floral morphs was observed as well as two flowering peaks (peak of the first flowering in October and December and the second in October and November) (Fig. 1AB). The peaks were separated by periods of 7-18 days without flower formation. No significant difference between the morphs were stated in terms of the average number of inflorescences/plant, flowers/inflorescence and open flowers/plant/day in both flowering seasons (Table 1). Despite the flowering synchrony of the morphs and the similarity between them in the mean values of the previous parameters, there was a trend of one morph to produce more flowers/peak/year (Table 1). There was no significant difference between flowers/morph/peak in the peaks of 2004 and 2005. However, significant differences were recorded in the total number of flowers per morph in the first flowering and no difference in the second. This variation may be related to the number of plants sampled in each flowering season (Table 1).

In the first flowering, the first flowers of *Psychotria conjugens* (Fig. 1CD) were observed in November in both years overlapping with the flowering of *P. sessilis*. The flowering periods ended in January 2005 and December 2005, respectively (Fig. 1CD), also during the rainy season. In the first reproductive episode, there were two peaks, in November and in January, with a drop in flower production of both morphs between the peaks, similarly as reported for *P. sessilis*. In the second reproductive episode, however, there was only one flowering peak in December 2005 (Fig. 1D). In both flowering seasons, there was synchrony between floral morphs (Fig. 1CD). There were significant differences between the floral morphs of first flowering, the average number of inflorescences/plant (Table 1) and no significant difference for this parameter in the second flowering (Table 1).

In the flowering of 2004, the average number of flowers/inflorescence was significantly higher for the long-styled morph, as well as the average number of...
Figure 1 – Flowering of short-styled (○) and long-styled flower morphs (●) in species of Psychotria: P. sessilis, from September to December 2004 (A) and from September to November 2005 (B); P. conjugens, from November 2004 to January 2005 (C) and from November to December 2005 (D); and P. hastisepala, from January to April 2005 (E) and from January to April 2006 (F).
Flowering and pollinators of three distylous species of...

Table 1 – Mean numbers and standard deviation of inflorescences/plant, flowers/inflorescence and open flowers/plant/day and number of flowers/peak in two consecutive flowering seasons, in 2004/2005 and 2005/2006, of *Psychotria sessilis*, *P. conjugens* and *P. hastisepala* in Viçosa, southeastern Brazil.

| Year / No. of plant/morph | *Psychotria sessilis* | Flowers/peak |
|---------------------------|----------------------|--------------|
|                           | Infl./ plant | Flowers/ infl. | Open flowers/plant/day |                  |
|                           | $\bar{x} \pm SD$ | $\bar{x} \pm SD$ | $\bar{x} \pm SD$ | $1^o$ | $2^o$ |
| 2004/ | | | | | |
| S (19) | 51.34 ± 40.63 | 9.46 ± 2.64 | 20.40 ± 22.20 | 244 | 144 |
| L (23) | 68.08 ± 80.67 | 7.86 ± 1.56 | 21.30 ± 25.50 | 341 | 97 |
| F=0.60 | p=0.44 | F=2.56 | p=0.11 | F=0.54 | p=0.46 | F=1.22 | p=0.28 |
| Total number of flowers per peak | 585 | 241 |
| 2005/ | | | | | |
| S (10) | 40.00 ± 25.01 | - | 11.44 ± 14.80 | 174 | 116 |
| L (10) | 45.50 ± 44.79 | - | 07.03 ± 16.32 | 32 | 148 |
| F=0.26 | p=0.61 | F=0.29 | p=0.60 | F=1.90 | p=0.18 | F=2.23 | p=0.15 |
| Total number of flowers per peak | 206 | 264 |

| Year / No. of plant/morph | *Psychotria conjugens* | Flowers/peak |
|---------------------------|----------------------|--------------|
|                           | Infl./ plant | Flowers/ infl. | Open flowers/plant/day |                  |
|                           | $\bar{x} \pm SD$ | $\bar{x} \pm SD$ | $\bar{x} \pm SD$ | $1^o$ | $2^o$ |
| 2004/ | | | | | |
| S (9) | 19.65 ± 1.06 | 12.30 ± 7.43 | 14.07 ± 5.70 | 139 | 42 |
| L (4) | 18.25 ± 3.50 | 22.68 ± 8.83 | 26.58 ± 6.80 | 181 | 28 |
| F=13.86 | p=0.004 | F=35.19 | p=0.0001 | F=8.15 | p=0.03 | F=26.50 | p=0.0001 | F=2.42 | p=0.22 |
| Total number of flowers per peak | 320 | 70 |
| 2005/ | | | | | |
| S (10) | 2.90 ± 2.51 | - | 4.22 ± 2.25 | 170 | - |
| L (10) | 3.30 ± 1.76 | - | 4.44 ± 3.51 | 184 | - |
| F=0.16 | p=0.68 | F=2.23 | p=0.14 | F=17.84 | p=0.23 |
| Total number of flowers per peak | 354 | - |

| Year / No. of plant/morph | *Psychotria hastisepala* | Flowers/peak |
|---------------------------|----------------------|--------------|
|                           | Infl./ plant | Flowers/ infl. | Open flowers/plant/day |                  |
|                           | $\bar{x} \pm SD$ | $\bar{x} \pm SD$ | $\bar{x} \pm SD$ | $\bar{x} \pm SD$ |
| 2005/ | | | | | |
| S (22) | 14.20 ± 06.54 | 6.91 ± 2.77 | 2.78 ± 2.41 | 410 |
| L (18) | 15.27 ± 15.10 | 9.03 ± 1.88 | 2.75 ± 2.28 | 362 |
| F=0.28 | p=0.56 | F=2.44 | p=0.12 | F=1.06 | p=0.30 | F=1.06 | p=0.30 |
| Total number of flowers | 732 |
| 2006/ | | | | | |
| S (10) | 17.10 ± 4.09 | - | 2.14 ± 2.70 | 193 |
| L (10) | 13.30 ± 5.33 | - | 1.35 ± 1.60 | 122 |
| F=4.28 | p=0.05 | F=5.60 | p=0.01 | F=5.62 | p=0.01 |
| Total number of flowers | 315 |
open flowers/plant/day. In the flowering of 2005, no
significant differences were observed for this last
parameter, although the average number of flowers/
inflorescence was similar. In the flowering of 2004, there
were significant differences in the number of flowers/
morph in the first peak; a larger number of long-styled
flowers were produced, while there was no difference in
the second peak (Table 1). The total number of flowers/
morph, in the first flowering, did not differ significantly,
however the flower production was significantly higher
in the first peak (F = 31.44, p = 0.0001). In the second
flowering, the flower production of both morphs was
similar (Table 1). At both flowering seasons, despite the
higher number of plants studied, there was a drop in
the production of inflorescences/plant and open flowers/
plant/day (Table 1).

Psychotria hastisepala initiated flowering in January
2005 and January 2006 (Fig. 1EF), also in the rainy season,
with little or no overlapping with the flowering of P.
conjugens. This species had the least abundant flowering
of all species studied. Both flowerings ended at the
beginning of the dry season.

Only one flowering peak was observed, from
February to March (Fig. 1EF), and the flowering of
the morphs was synchronous. There was no significant
difference between the morphs in the average number of
inflorescences/plant and flowers/inflorescence in
both flowering seasons (Table 1). In the flowering of
2005, there was no significant difference between the
morphs in the number of open flowers/plant/day, and
in 2006, this parameter was significantly higher in the
short-styled morph (Table 1). In the flowering of 2005,
the number of flowers/morph at the peak of flowering
was not significantly different, but differences were
observed in 2006 (Table 1). The production of flowers/
year was significantly higher in the flowering of 2005
(F = 16.53, p = 0.0006).

3.2. Flower visitors

Insects of the order Hymenoptera, Lepidoptera,
Diptera, and Coleoptera were recorded as flower visitors
on the species studied (Table 2). Among these, mainly
the native bees Ariphanarthra palpalis (Halictidae,
female and male), Melipona bicolor, M. mondary, M.
quadrispaciata (Apidae), and Trichocerapis mirabilis
(Apidae), and the exotic bee Apis mellifera were
considered pollinators because of their visit behavior
and visitation frequency (Table 2). During their visits,
in search of nectar, these bees inserted part of their
body into the floral tube and touched anthers and stigmas
in both morphs, promoting legitimate pollination. During
foraging bouts, generally all open flowers of a plant
were visited and, in this case, illegitimate, geitonogamous
pollination (sensu RICHARDS, 1997) tended to occur.
The flowers were visited mainly in the morning, between
7:00 and 10:00 h, continuing until 14:30, when the flowers
of all species became senescent.

On P. sessilis flowers, six bee species were observed
and three of them, M. quadrispaciata, M. bicolor and
Apis mellifera, were common in both flowering seasons
and played an important role as pollinators, because their
visitation frequency to both floral morphs was the highest
(Table 2). Melipona quadrispaciata and M. bicolor
accounted for 72.60 and 69.37% of the total number of
visits in the first (2004) and the second flowering (2005),
respectively (Table 2). The percentage of total visits of
the exotic bee A. mellifera was below 18.0% (Table 2).

The visits of other bees and one butterfly occurred
in only one flowering season and represented low
percentages (Table 2), except for Ariphanarthra palpalis;
this bee visited both morphs, accounting for about
15.0% of all visits, in the flowering of 2005. Long-styled
flowers tended to receive a greater number of visits;
in the second flowering in 2005, these flowers were
visited about 1.8 more times than the short-styled (Table 2).
On the 12 h observation/year for the counting of flower
visitors, insects were recorded on the flowers for 91.67%
of the time in the flowering of 2004, and for 75% in
2005.

Three bee species in common with P. sessilis were
observed on P. conjugens flowers (Table 2).
Ariphanarthra palpalis and M. bicolor were the most
important pollinators on both morphs (Table 2). These
bees were responsible for 87.32% of all visits. Melipona
quadrispaciata also served as pollinator of both morphs,
but at a low percentage (5.02% of total visits). The
frequency of visits of flies together with beetles were
unrepresentative (5.69%) compared to the bees (92.34%).
Long-styled flowers were visited nine times as much as
the short-styled (Table 2). On the 12 h observation
for the counting of flower visitors, insects on flowers
were recorded for 66.7% of the time.

Four bee species were observed on P. hastisepala
flowers (Table 2): A. palpalis and M. mondary in common
with P. sessilis and A. palpalis in common with P.
Flowering and pollinators of three distylos species of...

Table 2 – Frequency of visits of insects to the flowers *Psychotria sessilis*, *P. conjugens* and *P. hastisepala*, in two consecutive flowering seasons in Viçosa, southeastern Brazil.

| Insects (order/ family/ species) | Number of visits (%) |
|----------------------------------|----------------------|
|                                  | 2004                 | 2005                 |
|                                  | S    | L   | Total/ (% visits)        | S     | L   | Total/ (% visits)        |
| *Psychotria sessilis*            |      |     |                         |       |     |                         |
| Hymenoptera/ Apidae/             |      |     |                         |       |     |                         |
| *Apis mellifera* Lepeletier, 1836| (19.15) | (16.84) | 194 (17.90)           | (19.89) | (16.84) | 123 (17.90)         |
| *Melipona bicolor* Lepeletier, 1836| (29.03) | (11.73) | 213 (19.65)          | (29.83) | (11.73) | 334 (19.65)         |
| *Melipona monndury* Smith, 1863  | (05.85) | (06.97) | 70 (06.46)          |       |       |                         |
| *Melipona quadrifasciata* Lepelletier, 1836| (41.73) | (62.41) | 574 (52.95)      | (29.83) | (36.88) | 341 (34.33)         |
| Halictidae/ *Ariphanaartha palpalis*|       |     |                         |       |     |                         |
| Moure, 1951                      |       |     |                         |       |     |                         |
| *Augochlora* sp.                 |       |     |                         |       |     |                         |
| Lepidoptera/ Nymphalidae/         |       |     |                         |       |     |                         |
| Morph-species I                  |       |     |                         |       |     |                         |
| Total                            | 496  | 588 | 1084 (19.89)          | 352  | 621 | 973 (19.89)         |

| *Psychotria conjugens*            |      |     |                         |       |     |                         |
| Hymenoptera/ Apidae/             |      |     |                         |       |     |                         |
| *Melipona bicolor* Lepeletier, 1836|       |     |                         |       |     |                         |
| *Halictidae/ *Ariphanaartha palpalis* Moure, 1951|       |     |                         |       |     |                         |
| Diptera/Morph-species 1/          |       |     |                         |       |     |                         |
| Total                            | 76   | 680 | 756 (19.89)          |       |     |                         |

| *Psychotria hastisepala*         |      |     |                         |       |     |                         |
| Hymenoptera/ Apidae/             |      |     |                         |       |     |                         |
| *Melipona monndury* Smith, 1863  |       |     |                         |       |     |                         |
| *Trichocerapis mirabilis* Smith, 1865| (44.00) | (48.48) | 27 (46.55)   | (44.00) | (48.48) | 38 (48.48)         |
| *Halictidae/ *Ariphanaartha palpalis* Moure, 1951| (12.00) | (12.12) | 7 (12.00)     | (12.00) | (12.12) | 20 (12.00)         |
| *Augochlora* sp.                 |       |     |                         |       |     |                         |
| Morph-species I                  |       |     |                         |       |     |                         |
| Diptera/Morph-species 1/          |       |     |                         |       |     |                         |
| Total                            | 25   | 33  | 58 (12.00)          | 31   | 40  | 71 (12.00)         |

S = short-styled, L = long-styled
conjungens (Table 2). All bees were observed in only one flowering season. In the first flowering in 2005, *T. mirabilis* and *A. palpalis* were the most important pollinators on both morphs, accounting together for 74.55% of the total visits and in the second flowering in 2006, *M. montdury* and *Augochlora* sp. were the most important pollinators of both morphs, but accounted together for only 32.39% of all visits (Table 2). In the first flowering, one butterfly species was observed, and in the second a fly species, which accounted for 25.90 and 47.89% of all visits, respectively (Table 2). These insects, however, due to the small body size and/or visit behavior did not seem to act as pollinators; the same was true for the beetle observed in both flowerings (Table 2). These latter insects cut the stigmas of the long-styled flowers. The total number of visits/morph was similar in both flowerings, although the long-styled flowers received a greater number of visits (Table 2). On the 16 h observation/year for the counting of flower visitors, insects were recorded on flowers over 56.25% of the time in the flowering of 2005, and over 37.50% in 2006.

4. DISCUSSION

The flowering of each studied species was restricted to a few months of the year (annual flowering sensu NEWSTROM et al., 1994) and to the rainy season, when temperatures are higher. This pattern is similar to that reported for other *Psychotria* species (ALMEIDA; ALVES, 2000; MORELLATO et al., 2000; PEREIRA et al., 2006).

The flowering of the species occurred in a sequence, over eight months. This flowering behavior was also reported for *Psychotria nuda* and *Psychotria brasiliensis* (ALMEIDA; ALVES, 2000) and for *Psychotria birotula*, *Psychotria mapouriodes* and *Psychotria pubigera* (CASTRO; OLIVEIRA, 2002). The continuous availability of nectar by flowers of different species with similar characteristics is considered a strategy leading to the formation and retention of a “food image” by common pollinators (THOMPSON, 1980), minimizing the competition for pollinators among the species. The staggered flowerings of the *Psychotria* species seem to promote a “replacement series” (sensu MACIOR, 1971), by which the floral resources are available to pollinators for a long period of the year.

Two sequential peaks of flower production in the studied flowering seasons, mainly in *P. sessilis*, were also observed in *P. mapouriodes* (CASTRO; OLIVEIRA, 2002) and *P. tenuinervis* (RAMOS; SANTOS, 2006). However, the success is surely also related to the amount of flowers available and the visitation frequency of flowers of each morph and both tend to vary between flowering seasons, as showed here. The composition and abundance of pollinator species may vary among years, as observed in this study, and populations (VIEIRA; SHEPHERD, 2002). The flower attractiveness depends on the number of inflorescences, the number of flowers opened/day and the availability of floral resources seems to be one of the factors that determine the diversity of pollinators (HERRERA, 1991; DONALDSON et al., 2002). In fact, the highest numbers of visits/year to the flowers of the studied species, observed in *P. sessilis* and *P. conjungens*, corresponded to the highest number of open flowers/day and, probably, to the greatest availability of resources.

The higher frequency of visits to long-styled flowers of all species studied, but mainly of *P. conjungens*, was also recorded in other *Psychotria* species (CASTRO; OLIVEIRA, 2002). The greater attractiveness of one floral morph for pollinators may cause an asymmetrical and unidirectional pollen flow (BARRETT, 1992). It is known that the reproductive success of distylous species, including those studied here, depends on the gene flow between floral morphs, whose agents are the pollinators, since illegitimate pollinations normally result in incompatibility (CASTRO et al., 2004; SILVA et al., 2010). One result of this preference for long-styled flowers is the deposition of incompatible pollen, resulting in stigma occlusion, preventing the compatible grains from germinating (GANDERS, 1979), affecting the fruiting of the morph in question. Indeed, in the study area, the long-styled flowers of *P. conjungens* and, mainly, of *P. hastisepala* produced lower fruit set than the obtained in short-styled flowers (SILVA; VIEIRA, 2013; SILVA et al., 2014)

Among the pollinator bees of the studied *Psychotria* species, *Ariphanarthra palpalis* was a common pollinator. This species is solitary, restricted to the forest interior, active in the early morning hours and on cloudy days. In entomological collections, it is considered relatively rare and its host plants are unknown (G. A. MELO, 2007 – pers. comm.). Therefore, the observations of the use of the floral resources of the studied species by males and females of this bee are unprecedented data. It has a peculiar morphological characteristic, a long tongue, making its visits to the...
tubular flowers of *Psychotria* possible. It is known that seven other *Psychotria* species occur in the understory of this study area, which also bloom in the rainy season (PEREIRA et al., 2006). Could these bees depend on the floral resources of *Psychotria* species, characterizing them as oligolectic bees?

Other common pollinators to *Psychotria* species belong of the *Melipona* genus. These bees are social, have perennial colonies and visit several floral types belonging to different families (CRUZ et al., 2005). However, some studies have shown that the persistence of different species of this genus depends on forest habitats, as they were not found in open native or anthropogenic environments (SILVEIRA et al., 2002). Among the species collected in the study area, *Melipa bicolor* is presumably threatened in the state of Minas Gerais (CAMPOS, 1998). Aside from the dependence on forests, this species needs relatively large hollow trees for nesting (SILVEIRA et al., 2002). Similarly, the solitary bee *Trichoceraphis mirabilis*, observed on flowers of *P. hastisepala*, depends on forest habitats, which influences its geographical distribution (SILVEIRA et al., 2002). So, except for the introduced *Apis mellifera*, the pollinator bees require forest habitats, similarly to the studied *Psychotria* species, which grow in the forest understory.

5. CONCLUSION

The flowering of *Psychotria sessilis*, *P. conjugens* e *P. hastisepala* is staggered, following this sequence of species, over eight months and in the rainy season. In each species, the flowering of the morphs is synchronous. The species have a common pollinator, the solitary bee *Ariphanarthra palpalis*. Other native pollinating bees belong to the *Melipona* genus. The visitation frequencies of the pollinators to the floral morphs of each species differ between morphs and flowering season. There is a higher frequency of visits to long-styled flowers, compared to the short-styled flowers, of all species studied.

6. ACKNOWLEDGEMENTS

The authors are indebted to the Research Foundation of the state of Minas Gerais - FAPEMIG for the financial support and to the administration staff of the Mata do Paraíso for granting access to the study area. This work is part of the doctoral thesis presented by the first author in the postgraduate program in Botany of the Federal University of Viçosa.

7. REFERENCES

ALMEIDA, E. M.; ALVES, M. A. Fenologia de *Psychotria nuda* e *P. brasiliensis* (Rubiaceae) em uma área de Floresta Atlântica no sudeste do Brasil. *Acta Botânica Brasílica*, v.14, n.3, p.335-346, 2000.

ANDERSSON, L. A. A provisional checklist of Neotropical Rubiaceae. *Scripta Botanica Belgica*, v.1, n.1, p.1-119, 1992.

BARRETT, S. C. H. Heterostylos genetic polymorphisms: model system for evolutionary analysis. In: BARRETT, S. C. H. (Ed.). *Evolution and function of heterostyly*. New York: Springer; 1992. p.1-24.

CASTRO, C. C.; OLIVEIRA, P. E. Pollination biology of distylous Rubiaceae in the Atlantic Rain Forest, SE Brazil. *Plant Biology*, v.4, n.4, p.640-646, 2002.

CASTRO, C. C.; OLIVEIRA, P. E. A. M.; ALVES, M. C. Breeding system and floral morphometry of distylous *Psychotria* L. species in the Atlantic rain forest, SE Brazil. *Plant Biology*, v.6, n.4, p.755-760, 2004.

CAMPOS, L. A. O. *Melipona rufiventris* Lepeletier, 1836. In: MACHADO, A. B. M.; FONSECA, G. A. B.; MACHADO, R. B.; AGUIAR, L. M. S.; LINS, L. V. (Ed.) *Livro Vermelho das espécies ameaçadas de extinção da fauna de Minas Gerais*. Belo Horizonte: Biodiversitas, 1998. p.560-561.

COELHO, C. P.; BARBOSA, A. A. A. Biologia reprodutiva de *Psychotria poeppigiana* Müll. Arg. (Rubiaceae) em mata de galeria. *Acta Botânica Brasílica*, v.18, n.3, p. 481-489, 2004.

CRUZ, D. O.; FREITAS, B. M.; SILVA, L. A.; SILVA, E. M. S.; BOMFIM, I. G. A. Pollination efficiency of the stingless bee *Melipona subnitida* on greenhouse sweet pepper. *Pesquisa Agropecuária Brasileira*, v.40, n.12, p.1197-1201, 2005.
DONALDSON, J.; NANNI, I.; ZACHARIADES, C.; KEMPER, J. Effects of habitat fragmentation on pollinator diversity and plant reproductive success in renosterveld shrublands of South Africa. Conservation Biology, v.16, n.5, p.1267-1276, 2002.

GANDERS, F.R. The biology of heterostyly. New Zealand Journal of Botany, v.17, n.4, p.607-635, 1979.

HERRERA, C.M. Dissecting factors responsible for individual variation in plant fecundity. Ecology, v.72, n.4, p.1436-1448, 1991.

KARRON, J.D.; MITCHELL, R. J. Effects of floral display size on male and female reproductive success in Mimulus ringens. Annals of Botany, v.109, n.3, p.563-570, 2012.

MACIOR, L.W. Coevolution of plants and animals - systematic insights from plant-insect interactions. Taxon, v.20, n.1, p.17-28, 1971.

MARTIN-GAJARDO, I.; MORELLATO, L.P.C. Fenologia de Rubiaceae do sub-bosque em floresta Atlântica no sul de trópicos do Brasil. Revista Brasileira de Botânica, v.26, n.3, p.299-309, 2003.

MORELLATO, L.P.C.; TALORA, D.C.; TAKAHASI, A.; BENCKE, C.C.; ROMERA, E.C.; ZIPPAR, V.B. Phenology of Atlantic rain forest trees: a comparative study. Biotropica, v.32, n.4b, p.811-823, 2000.

NEWSTROM, L.E.; FRANKIE, G.W.; BAKER, H.G. A new classification for plant phenology based on flowering patterns in lowland tropical rains forest trees, at La Selva, Costa Rica. Biotropica, v.26, n.2, p.141-159, 1994.

PENHALBER, E.F.; MANTOVANI, W. Floração e chuva de sementes em mata secundária em São Paulo, SP. Revista Brasileira de Botânica, v.20, n.2, p.205-220, 1997.

PEREIRA, Z.V.; VIEIRA, M.F.; CARVALHO-OKANO, R.M. Fenologia de floração, morfologia floral e sistema de incompatibilidade em espécies distílicas de Rubiaceae em fragmento florestal do sudeste brasileiro. Revista Brasileira de Botânica, v.29, n.3, p.471-480, 2006.

PRIMACK, R.B. Longevity of individual flowers. Annual Review of Ecology and Systematics, v.16, n.1, p.15-37, 1985.

RAMOS, F.N.; SANTOS, F.A.M. Floral visitors and pollination of Psychotria tenuinervis (Rubiaceae): Distance from the anthropogenic and natural edges of Atlantic Forest Fragment. Biotropica, v.38, n.3, p.383-389, 2006.

RICHARDS, A.J. Plant breeding systems. London: Chapman & Hall, 1997. 529p.

SAKAI, S.; WRIGHT, S.J. Reproductive ecology of 21 coexisting Psychotria species (Rubiaceae): when is heterostyly lost?. Biological Journal of the Linnean Society, v.93, n.1, p.125-134, 2008.

SILVA, C.A.; VIEIRA, M.F. Sucesso reprodutivo de espécies distílicas de Psychotria (Rubiaceae) em sub-bosque de Floresta Atlântica. Revista Árvore, v.37, n.2, p.289-297, 2013.

SILVA, C.A.; VIEIRA, M.F.; AMARAL, C.H. Floral attributes, ornithophily and reproductive success of Palicourea longipendunculata (Rubiaceae), a distylos shrub in southeastern Brazil. Revista Brasileira de Botânica, v.33, n.2, p.207-213, 2010.

SILVA, C.A.; VIEIRA, M.F.; CARVALHO-OKANO, R.M.; OLIVEIRA, L.O. Reproductive success and genetic diversity of Psychotria hastisepala (Rubiaceae), in fragmented Atlantic forest (Southeastern Brazil). Revista de Biologia Tropical, v.62, n.1, p.309-319, 2014.

SILVEIRA, F.A.; MELO, G.A.R.; ALMEIDA, E.A.B. Abelhas Brasileiras: sistemática e identificação. Belo Horizonte Fundação Araucária, 2002. 254p.

STATSOFT, INC. Statistica for windows (Computer Program Manual). Tulsa: StatSoft INC., 2002.

TAYLOR, C.; GOMES, M.; ZAPPI, D. Psychotria in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Disponível em: <http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB14211/>. Acesso em: 25 Abr. 2015.
THOMPSON, J.D. Skewed flowering distributions and pollinator attraction. Ecology, v.61, n.3, p. 572-579, 1980.

VELOSO, H.P.; RANGEL-FILHO, A.L.R.; LIMA, J.C.A. Classificação da vegetação brasileira, adaptada a um sistema universal. Rio de Janeiro: IBGE, 1991. 124 p.

VIEIRA, M.F.; SHEPHERD, G.J. Removal and insertion of pollinia in flowers of Oxypetalum (Asclepiadaceae) in southeastern Brazil. Revista de Biologia Tropical, v.50, n.1, p.37-43, 2002.

ZAR, J.H. Biostatistical analysis. 4.ed. New Jersey: Prentice Hall, 1999. 929 p.