

Differentiation of floral color and odor in two fly pollinated species of *Metrodorea* (Rutaceae) from Brazil

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Abstract. We investigated if differences in morphological characters in two species of *Metrodorea* (Rutaceae) from Brazilian semideciduous forests correspond to some pollination divergence. *M. nigra* and *M. stipularis* are sympatric species, display a similar floral morphology, are protandrous, self-incompatible, their flower periods overlap, and both are pollinated by flies. *M. nigra* main pollinators are *Pseudaletia nigripoda* (Muscidae) and *Fannia* sp. (Fanniidae); *M. stipularis* major pollinators are *Phaenicia eximia* (Calliphoridae), *Palpada* sp. and *Ornidia obesa* (Syrphidae). The distinct floral odor (disagreeable in *M. nigra* and sweet in *M. stipularis*) and color (brownish violet vs. pale yellow) determine the differences on type and number of floral visitors observed. Several species from semideciduous forests initially considered to be pollinated by diverse insects, present flies as main pollinators, stressing the importance of fly pollination in such habitats.

Key words: Brazil, Calliphoridae, dichogamy, Fanniidae, floral biology, *Metrodorea*, Muscidae, myiophily, phenology, pollination, Rutaceae, semideciduous forest, Syrphidae, tropical forest.

The diverse floral characteristics found among angiosperms have been interpreted as adaptive mechanisms related to a greater efficiency in pollination (Percival 1965, Baker and Hurd 1968, Faegri and Van Der Pijl 1979, Crepet

1983, Endress 1994). Plants have been grouped according to the presence of several floral features connected with the adaptation to some pollen vectors, the so-called pollination syndromes (Faegri and Van Der Pijl 1979). These syndromes or pollination systems have been regarded as an important pre-mating isolation mechanism, allowing for the co-occurrence of closely related species and potentially leading to speciation (Grant 1949, 1994; Baker 1963; Proctor et al. 1996; Waser et al. 1996).

In order to understand how a pollination system diverges between related species, a comparative study was developed with regard to the pollination ecology and reproduction of two sympatric species of *Metrodorea* (Rutaceae), *M. nigra* Saint-Hilaire and *M. stipularis* Martius, from semideciduous forests of southeastern Brazil. We want to investigate if differences in morphological characters in these two species will correspond to some pollination divergence. *Metrodorea* is a largely Brazilian genus of trees comprising only five species (Kaastra 1982) with a very conservative flower morphology. *M. nigra* is the only species with dark purplish-brown flowers with no recorded fragrance although we have detected a very unpleasant odor. *M. stipularis*

and the other species as well, show yellowish-white flowers, generally fragrant and considered as pollinated by "small diverse insects" (Kaastra 1982, Bawa et al. 1985).

We verify the occurrence of dichogamy and pollination by flies for both species of *Metrodorea*. We discuss the roles the differences on color and odor play in selecting distinct flower visitors for each species and the importance of fly pollination in neotropical seasonal forests.

Materials and methods

Study sites. The experimental work on the pollination ecology of *Metrodorea nigra* and *M. stipularis* was carried out in two areas of seasonal semideciduous forest (cf. Leitão Filho and Morellato 1997) separated by about 80 km, in the State of São Paulo, southeastern Brazil, during the 1991 to 1993 flowering seasons. Most of the field studies were conducted at the Santa Genebra Municipal Reserve (SGR), a 250 ha forest surrounded by soya fields and some houses, in the Campinas Municipality (22°49'S; 47°06'W; 670 m altitude). The Fazenda São José (FSJ) is a private farm located between Rio Claro and Araras Municipalities (22°22'S; 47°28'W; 630 m altitude) and the disturbed forest of about 230 ha is encircled by sugar cane crops. Only some of the breeding tests on *M. nigra* were performed at this FSJ forest.

The semideciduous forest is characterized by a canopy 15 to 20 m high, some emergent trees reaching up to 30 m tall and the presence of several species of lianas (Morellato and Leitão Filho 1995, Leitão Filho and Morellato 1997). The most species-rich tree families are Leguminosae, Meliaceae, Rutaceae, Lauraceae, Euphorbiaceae, and Rubiaceae (Pagano and Leitão Filho 1987, Morellato and Leitão Filho 1995). The general climate is tropical seasonal with a dry and cold season from April to September, when the precipitation is below 60 mm per month, and a wet and hot season from October to March when the rainfall is above 100 mm per month (Setzer 1946). The annual mean temperature is 21 °C and the annual mean precipitation is 1360 mm (Pombal 1994).

Voucher specimens of the plants investigated are deposited in the Herbarium Rioclarense (HRCB) and Herbário da Universidade Estadual de Campinas (UEC).

Study species. *Metrodorea nigra* is a small tree frequently found in the understorey of semideciduous forests (Pagano and Leitão Filho 1987). The observed trees and treelets were 2–6 m high, located along the forest edges, having flowers that are brownish violet (or dark purple according Kaastra 1982), small, pentamerous, with radial symmetry, open type, 9.4–12.0 mm in diameter ($x = 10.93$; $SD = 0.78$; $N = 10$), arranged in paniculated terminal inflorescences with 83.67 mm mean length ($SD = 34.35$; $N = 15$) (Fig. 1a). Petals are dark brownish violet, upper side densely pubescent. Stamen filaments 5, free, ca. 1.0 mm long, brownish violet, inserted on the edge of the receptacle (Fig. 1a), filament portions above the receptacle have the same lengths as the receptive stigma (about 0.6 mm); anthers are heart-shaped, papillose, versatile, dorsifixed, having longitudinal dehiscence, yellow with finely pink pigmentation (Fig. 1a); pollen grains are yellow and agglutinated. Styles are brownish violet, ca. 0.3 mm or 0.6 mm long when bearing an immature or a receptive stigma, respectively; stigmas are capitate, slightly lobed, green, presenting a fluid secretion when receptive (Fig. 1a). Receptacles contain ovoid, brownish violet, glandular tubercles; nectary disc of the same color, slightly lobed, about 2.6 mm diameter, located on the upper edge of the receptacle, surrounding the ovary (Fig. 1a). Fruits are capsules, 2 seeds per loci, becoming light brown when ripe.

Metrodorea stipularis is a canopy tree characteristic of the Brazilian semideciduous forests (Pagano and Leitão Filho 1987). The observed trees were 2.5–12.0 m high and occur along forest edges of the Santa Genebra Reserve, having flowers that are pale yellow, very small, pentamerous, open type with radial symmetry, 6.0–6.9 mm diameter ($x = 6.44$; $SD = 0.26$; $N = 10$), arranged in paniculate terminal inflorescences 225.91 mm mean length ($SD = 45.60$; $N = 11$) (Fig. 1c). Petals are pale yellow, minutely pubescent on both surfaces. Stamen filaments 5, free, ca. 1.5 mm long, pale yellow, laterally inserted on the receptacle (Fig. 1c); filament portions above the receptacle have the same lengths as the receptive stigma (ca. 0.9 mm); anthers are heart-shaped, versatile, having longitudinal dehiscence, dark pink color when in the flower buds and brownish yellow after the anthesis; pollen grains are yellow and agglutinated (Fig. 1c). Styles are light green, ca. 0.5 mm or 1.0 mm long



Fig. 1. The dichogamic flowers and flower visitors of *Metrodorea*. **a** and **b** Flowers of *Metrodorea nigra* (3×). **a** Flowering sequence from male to female phases; observe the flower in the male phase with an opaque stigma and the nectar at the base of the petals (opaque color), and the female phase with a shiny stigma. **b** *Pseudoptiloleps nigripoda* visiting a flower of *Metrodorea nigra* in the male phase; note the pollen grains adhering to the tongue (arrow). **c** and **d** Flowers of *Metrodorea stipularis*. **c** Flowers in the male phase with opaque stigmas; beginning and end of the female phase with opaque and shiny stigmas, respectively (5×); note the change in color of the nectariferous disk from yellow (*M*) to light pink (*F1*), to reddish (*F2*). **d** *Phaenicia eximia* visiting a flower of *Metrodorea stipularis*; note the position of the tongue relative to the reproductive organs of the flower (3×). Abbreviations: *M* male phase; *F* female phase; *F1* flowers in the female phase with opaque stigmas; *F2* the female phase with shiny stigmas

when bearing an immature or a receptive stigma, respectively; stigmas are capitate, dark green, presenting a fluid secretion when receptive (Fig. 1c). Receptacles contain several pale yellow, glabrous, glandular tubercles around the style; nectary disc 5-lobed, about 2.0 mm diameter, located on the upper edge of the receptacle, surrounding the ovary. The nectary disc shows a yellow color just after the antheses (one or two days), gradually turning rose to light red as the antheses proceeds (second to fifth day; Fig. 1c). Fruits are capsules with 2 seeds per loci becoming light brown when ripe.

Phenology. Phenological observations were conducted weekly during the flowering season to estimate the mean numbers of flowers in the male and female phases and of fruits produced. Fourteen inflorescences from seven individual trees of *M. nigra* were marked and the number of flower buds, open flowers and fruits produced were counted weekly in the field in 1992. Due to the small size and the large number of flowers produced by *M. stipularis*, four inflorescences of five marked trees were collected randomly each week during the 1993 flowering season and the buds/flowers produced were counted in the laboratory

under a stereomicroscope. In order to estimate the number of fruits produced per inflorescence, 10 inflorescences of *M. stipularis* were marked in the field and were observed weekly for fruit production. General phenological observations were conducted in the three flowering seasons.

Floral biology. Periodic observations on the floral biology and morphology of *M. nigra* and *M. stipularis* were carried out throughout the different stages of development for 50 flowers of each species. Data about the time of anthesis, receptivity of the stigma, availability and viability of the pollen, duration of the flower, and concentration of sugars in the nectar were recorded. We also verified if lipid substances were present in the pollen grains and in droplets removed from the wall of the anther and from the glandular tubercles. The coloration and odor of the flowers, as well as the type of resource offered to the visitors, were recorded.

The receptivity of the stigma was tested with glycerinated SUDAN III and hydrogen peroxide (Zeisler 1938, Johansen 1940), and further confirmed using the shiny stigma method (Percival 1965). The viability of the pollen was verified in flower buds prior to anthesis and in flowers during the male phase. In both cases we counted 400 pollen grains using the technique of malachite green and fuchsin acid (Alexander 1980). We measured the concentration of sugars in the nectar with a pocket refractometer. For the perception of odor, several flowers of each species were conditioned in distinct glass containers. The color of the flowers was determined according to Kornerup and Wanscher (1963). To test for the presence of lipid we used the methods of SUDAN – alcohol solution 0.1%, lugol solution and methylene blue. In addition, droplets removed from the glandular tubercles and the external wall of the anther were placed on a glass slide and maintained exposed to the air in order to verify if they had dried out after a few days.

Breeding systems. Four types of hand-pollination experiments were conducted in the field using paper-bagged inflorescences to determine the reproductive system of the *Metrodorea* species. (a) Spontaneous self-pollination – flower buds were marked with labels and the whole inflorescence was covered with a paper bag; (b) Manual self-pollination – hand self-pollinated flowers were paper-bagged; (c) agamospermy – flower buds were

emasculated, marked and bagged; (d) cross-pollination – pollen of different individuals was transferred to the stigma of flowers that had been previously emasculated and the flowers were bagged. Forceps were used to emasculate the flower buds, by perforating the corolla to remove the anthers. Individual flowers in otherwise non-treated inflorescences were tagged and left uncovered to assess natural fruit set.

Flower visitors. The time and frequency of visits to the flowers of *M. nigra* and *M. stipularis* were registered in two non-consecutive days, through fifteen-minute-long collections, undertaken every hour during the whole day, in one individual from each species. The observations were finished after one hour with no visitors observed. We analysed the relationship between the number of visitors and the relative humidity of the air or temperature using the Spearman rank correlation test (Zar 1984). To study the behavior and the type of resource used by the floral visitors we made direct visual observations that were complemented with photographs and VCR videotapes. General observations of flower visitors were conducted throughout the flowering seasons of the two species at the SGR.

Voucher specimens of the animals were deposited at the Museu de História Natural da Universidade Estadual de Campinas (ZUEC).

Results

Phenology. *Metrodorea nigra* flowered in October and November and *M. stipularis* bloomed in November and December during the three years of study (1991–1993). Morellato (1991) observed the same annual pattern in 1989 and 1990 for both species at SGR, with *M. stipularis* flower season extending until January in 1990. Both species presented seed dispersal by explosive opening of the capsules at the end of the dry season, in September. The flowering of the two species of *Metrodorea* overlapped during November, and their flower peaks were separated by two weeks (Fig. 2a, b). Nevertheless, there was no significant difference between flower times (Mann–Whitney test, $U = 66$, $p = 0.241$). We found a larger number of flowers in the female phase

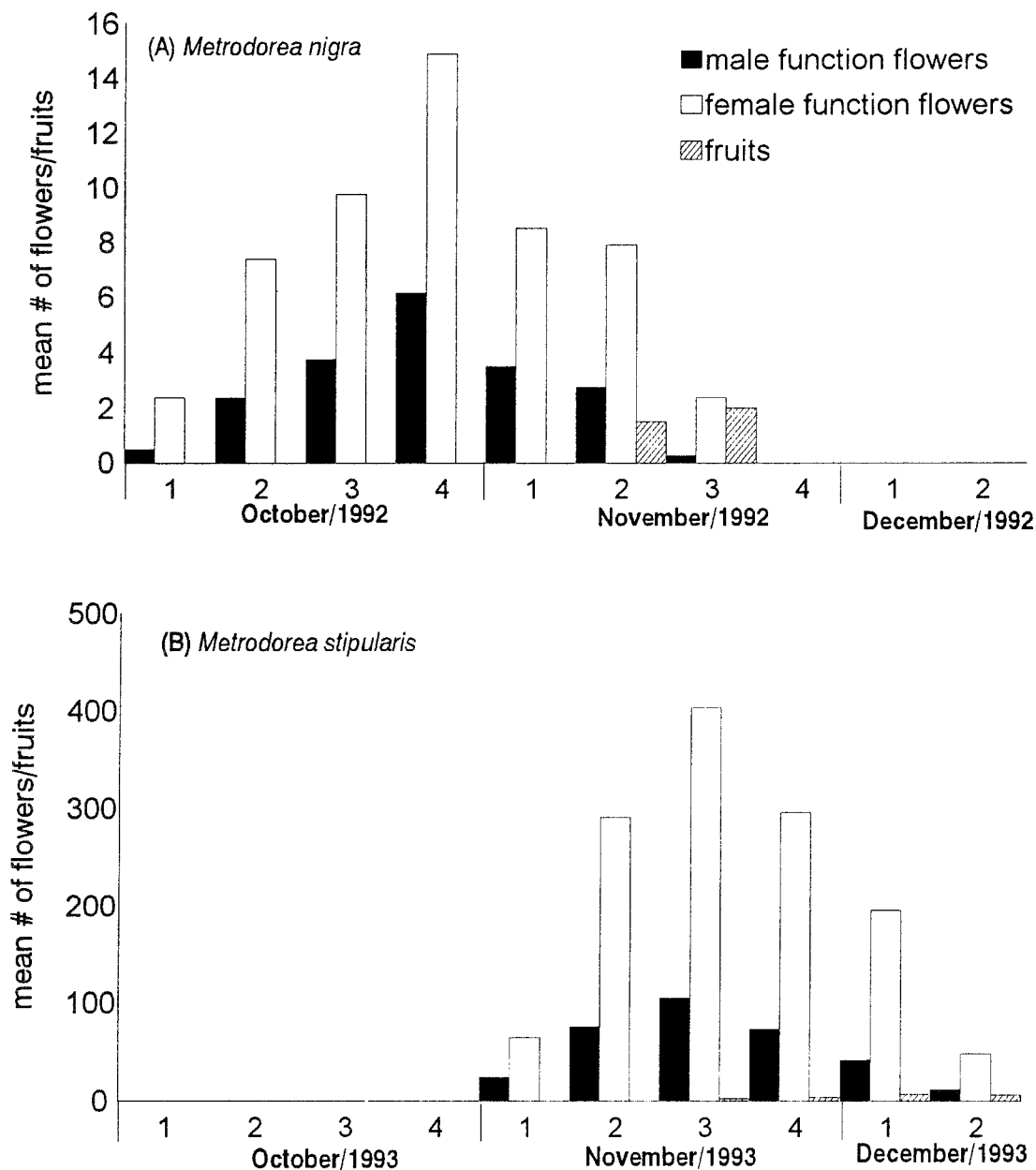


Fig. 2. Mean flower production and the onset of fructification for **A** *Metrodorea nigra*, and **B** *Metrodorea stipularis*. Numbers 1 to 4 represent the weeks of each month

than in the male phase due to the longer duration of the female phase (Fig. 2a, b). The mean number of flower and fruits produced per inflorescence, during the reproductive season of *M. nigra* was 67.36 (SD = 40.08; n = 14) and 1.07 (SD = 1.27; n = 14), respectively. The proportion of flower:fruits was about 63:1. The mean number of flower and

fruits produced per inflorescence by *M. stipularis* was 1190.5 (SD = 257.26; n = 4) and 5.0 (SD = 4.42; n = 10) respectively, with an average flower:fruit proportion of 238:1.

Floral biology. The floral biology of both species of *Metrodorea* studied was essentially the same, differing only in the time of anthesis and the flower odor. Anthesis in *M. nigra*

started at ca. 16:00 h and, after opening and in the first few hours of the day until about 07:00 h, a slight disagreeable odor was noticed emanating from the flowers. The anthesis of *M. stipularis* started at about 05:00 h in the morning. We noticed a strong, agreeable and sweet odor throughout the day on the flowers and in the proximity of the plants of *M. stipularis*. The number of open flowers (functional flowers) per inflorescence per day ranged from 1–33 ($x = 7.95$; $SD = 6.62$; $N = 42$) in *M. nigra* and from 110–249 ($x = 164.80$; $SD = 53.49$; $N = 5$) *M. stipularis*.

Anthesis commenced slowly in both species, beginning with the slackening of the petals that remained united only by the apex. Next, the petals detached from the flower apex and the completion of opening occurred after approximately two hours. Total anthesis duration was about five days. In the first day it was in the male phase and from the second to fifth day, in the female phase (Fig. 1a, c). The flower in the male phase produces nectar, and the pollen is viable and exposed to the visitors (Fig. 1a, 1c, male phase); the undeveloped style presents an opaque, non-receptive stigma. In the female phase, the flower has a well-developed style with a shiny receptive stigmatic area, and the production of nectar continues; the filaments are curved towards the petals, the anthers are located away from the center of the flower and there is practically no pollen (Fig. 1a, c). The nectar is secreted in small quantities during the whole period of anthesis and accumulates in the posterior region of the receptacle and, in *M. nigra*, it also accumulates at the base of the petals (Fig. 1a). In *M. stipularis*, after the period of anthesis, even if the flower has not been fertilized, the petals remain attached to the flower for a few days, although nectar is no longer produced and the nectariferous disk has a reddish coloration (Fig. 1c). Next, the flower wilts and detaches itself from the inflorescence. The average sugar concentration in the nectar of *M. nigra* flower-bud prior to anthesis was 4.75% ($SD = 0.76$; $N = 6$). The average sugar concentration in *M. stipularis* nectar was 6.90% ($SD = 0.99$;

$N = 4$) and 18.12% ($SD = 5.22$; $N = 5$), for the male and female phases, respectively.

In both species, stigma receptivity was confirmed by the secretion emitted from the stigmatic region that caused it to become shiny, and by the other two methods used. The viability of the pollen grains in both species was 100%, both in the flower bud before anthesis and in the flower in the male phase. The anthers presented an accumulation of droplets of a sticky substance that adhered to the external wall parallel to the dehiscence of the anthers. These droplets reacted positive for the presence of lipids. The glandular tubercles also produced a lipid substance, secreted only when pressed. The pollen grains agglutinated due to the presence of lipid substances, constituting a “pollenkitt” (cf. Dobson 1988).

Breeding systems. The reproductive system experiments showed that both *Metrodorea* species are self-incompatible and produce fruits only by cross-pollination (Table 1).

Flower visitors. Flies were the most frequent visitors to both species of *Metrodorea* studied, and nectar was the main floral resource searched for by these visitors. *Metrodorea nigra* was visited exclusively by eight families of Diptera (Table 2) whereas *M. stipularis* was visited by eight families of Diptera and a few Hymenoptera during the study period (Table 3). The same species of flower visitors were observed during the qualitative field observations.

Metrodorea nigra. The flies exhibited two activity periods on *M. nigra* (Fig. 3a). In the morning period they began their visits at about 05:00 h, prolonging it until 09:00 h. In the afternoon, they began the visits after the opening of the flowers, around 16:00 h, extending it until 18:00 h. During this period the frequency of flower visits was less than in the morning (Fig. 3a). No visits occurred between 09:00 and 16:00 h or after 18:00 h. There was a negative correlation between the air temperature ($r_s = -0.7006$; $n = 12$; $p < 0.05$) and the number of visitors.

The Muscidae flies, represented by a single species, *Pseudoptiloleps nigripoda* (Fig. 1b),

Table 1. Results of controlled pollination experiments for two species of *Metrodorea* (Rutaceae)

Treatments	<i>M. nigra</i>			<i>M. stipularis</i>		
	n° of Flowers	n° of Fruits	Success %	n° of Flowers	n° of Fruits	Success %
Spontaneous self-pollination	78	0	0	34	0	0
Manual self-pollination	77	0	0	24	0	0
Cross pollination	54	14	26	14	5	36
Agamospermy	83	0	0	120	0	0
Control	136	8	6	100	16	16

Table 2. Species and frequency of flower visitors on the flowers of *Metrodorea nigra* (Rutaceae)

Family	Species	Frequency ^a
Muscidae	<i>Pseudauleps nigripoda</i>	frequent
Fanniidae	<i>Fannia</i> sp.	frequent
Mycetophilidae	sp. 1	rare
	sp. 2	rare
Sciaridae	sp. 1	rare
	sp. 2	rare
Lauxaniidae	sp. 1	rare
	sp. 2	rare
Calliphoridae	<i>Chloroprocta</i> sp.	rare
Syrphidae	<i>Palpada</i> sp.	rare
Drosophilidae	<i>Drosophila</i> sp.	rare

^a The frequency of visits is based on the number of flower visitors collected during 15 min each hour on two non-consecutive days (see methodology for more details). Frequent: number of visits/day $n > 10$, rare: $n < 10$

were the most frequent visitors to the flowers of *M. nigra*. The greatest frequency of visits occurred in the morning between 05:00 and 07:00 h (Fig. 3a). The second most important flower visitors were individuals of a single species, *Fannia* sp. (Fanniidae). The visits were most frequent in the afternoon, from 17:00 to 18:00 h (Fig. 3a). The visit behavior of *Fannia* sp. was similar to the one described below for *P. nigripoda* (Muscidae). The visits of other families of flies to *M. nigra* were rare and occurred at variable times (Fig. 3a, Table 2).

The *P. nigripoda* flies landed on the petals of the flower they were about to visit and with their tongue touched the receptacle and the base of the petals several times, licking the nectar that had accumulated. Sometimes the flies would support themselves on neighboring flowers of *M. nigra*. While licking the nectar

from the receptacle of the male-phase flowers, the fly tongue would contact the anthers and pollen grains would adhere to it (Figs. 1b and 4). The tongue would contact the substance accumulated on the external wall of the anthers as well, and the end of the tongue probably pressed the receptacle glandular tubercles, releasing the lipid substance contained in them (Fig. 4). When visiting the female-phase flowers, the fly tongue contacted the stigma while removing the nectar. The flies would visit practically all the flowers of an inflorescence before moving to another. The families Calliphoridae and Syrphidae, represented by the species *Chloroprocta* sp. and *Palpada* sp.1 presented a visiting behavior similar to the one observed for Muscidae. However, they rarely visited the flowers of *M. nigra* (one individual of each species was

Table 3. Species and frequency of flower visitors on the flowers of *Metrodorea stipularis* (Rutaceae)

Family	Species	Frequency ^a
Calliphoridae	<i>Phaenicia eximia</i>	frequent
	<i>Chrysomia putoria</i>	eventual
	<i>Chrysomia megacephala</i>	eventual
	<i>Hemilucilia segmentaria</i>	rare
Syrphidae	<i>Ornidia obesa</i>	frequent
	<i>Palpada</i> sp.1	frequent
	<i>Palpada furcata</i>	frequent
	<i>Toxomerus taenia</i>	eventual
	<i>Allograta colombia</i>	eventual
	<i>Toxomerus</i> sp.	eventual
	<i>Palpada vinetorium</i>	rare
	<i>Oxyptamus</i> sp.	rare
	<i>Palpada geniculata</i>	rare
	<i>Palpada</i> sp.2	rare
	<i>Allograta</i> sp.	rare
	Sarcophagidae	<i>Helicobia</i> sp.
<i>Lipoptilocnema</i> sp.		rare
<i>Oxysarcodexia</i> sp.		rare
<i>Oxysarcodexia thornax</i>		rare
<i>Sarcodexia</i> sp.		rare
Tachinidae	Goniinae eryciini	eventual
	Phasiinae phasiini	eventual
	Trichopodini	eventual
	Goniinae harrisiini	eventual
	Cylindronyini	eventual
	Tachininae dejeaniini	rare
	Proseninae theresiini	rare
	Dexiinae leskiini	rare
	Dexiinae telothyriini	rare
	Goniinae belvosiini	rare
	Goniinae masiphyini	rare
	Tachininae juriniini	rare
	Muscidae	<i>Musca domestica</i>
<i>Biopyrellia bipuncta</i>		rare
Stratiomidae	sp.	rare
Lauxaniidae	sp.	rare
Tabanidae	sp.	rare
Vespidae	<i>Polistes versicolor</i>	eventual
	<i>Mischocyttarus drewseni</i>	eventual
	<i>Polybia sericea</i>	rare
	<i>Protoectaria sylveirae</i>	rare
	<i>Polybia ignobilis</i>	rare
	<i>Polybia paulista</i>	rare
	<i>Polybia jurinei</i>	rare
	<i>Mischocyttarus cassununga</i>	rare
Pompiliidae	sp.	rare
Eumenidae	sp.	rare

^a The frequency of visits is based on the number of flower visitors collected during 15 min each hour on two non-consecutive days (see methodology for more details). Frequent: number of visits/day $n > 12$, eventual $5 < n < 12$, and rare: $n < 5$

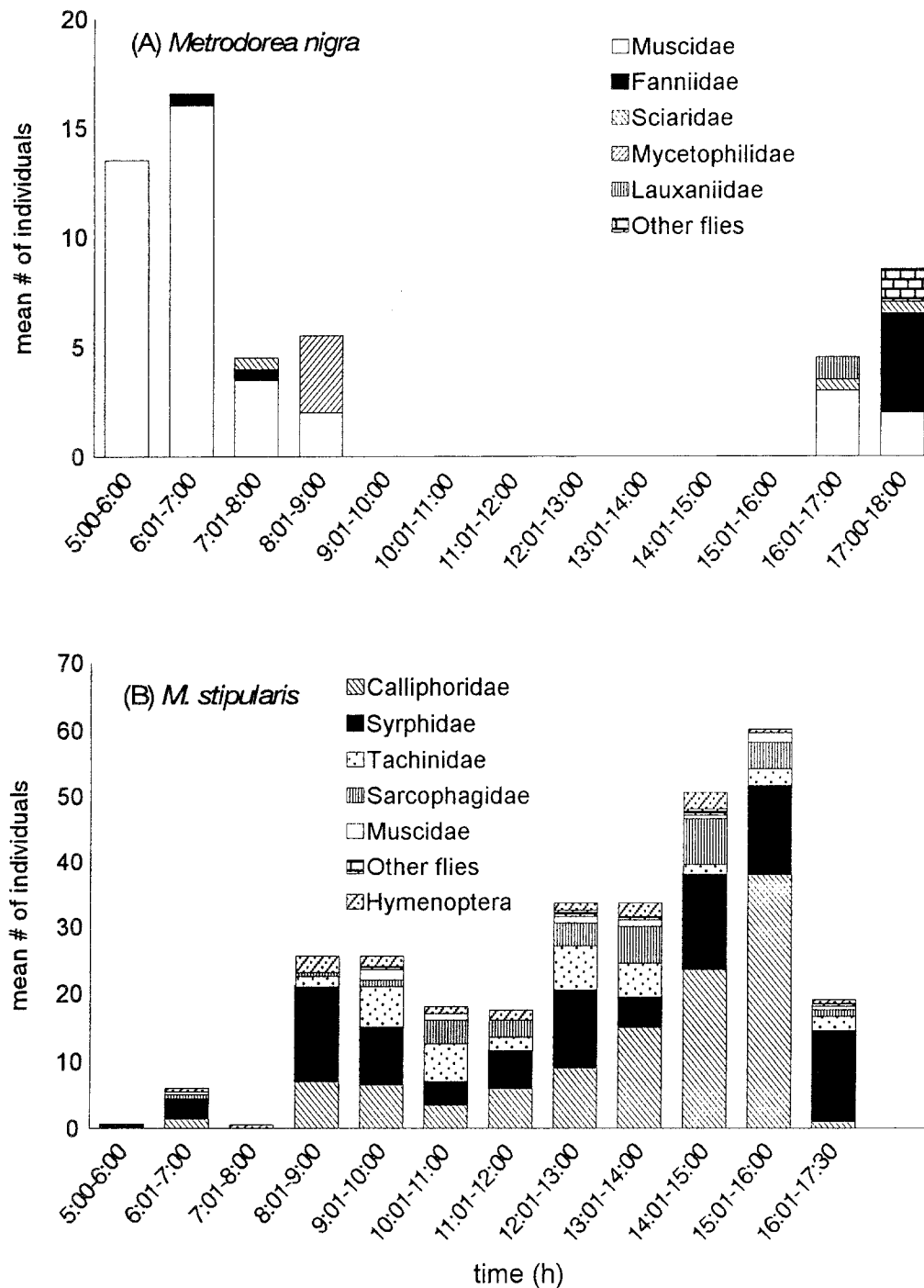


Fig. 3. Time and frequency of flower visitors (from several families of Diptera and Hymenoptera) to the flowers of **A** *Metrodorea nigra* and **B** *Metrodorea stipularis* throughout the day

observed, Fig. 3a), and they were classified as secondary pollinators (rare in Table 2). Flies from the families Mycetophilidae, Sciaridae, Lauxaniidae and Drosophilidae were consid-

ered to be nectar robbers (sensu Inouye 1980), since they were small and removed nectar without touching the reproductive organs of the flower.

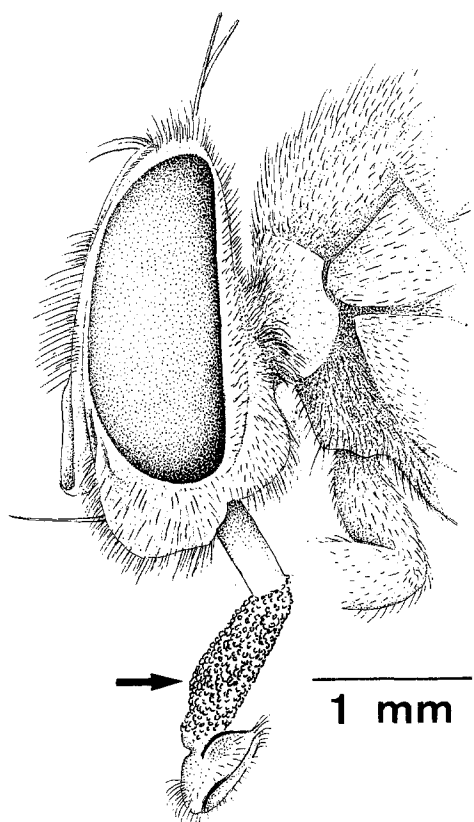


Fig. 4. Illustration of part of a fly showing the region on the tongue where the pollen grains become attached (arrow)

The analysis of the visit behavior associated to the floral morphology of *M. nigra* shows that all the visitors, with the exception of the small flies, are able to pollinate this species. However, according to the visitation frequency, the main pollinators of *M. nigra* are the flies *Fannia* sp. and, especially, *Pseudoptiloleps nigripoda*, from the families Fanniidae and Muscidae, respectively.

Metrodorea stipularis. The Diptera and Hymenoptera visits to *M. stipularis* flowers began around 05:30 h and extended to 17:30 h (Fig. 3b). There was a significant positive correlation between the air temperature and the number of visitors ($r_s = 0.8561$; $N = 12$; $p < 0.01$), and a significant negative correlation between the air humidity and the number of visitors ($r_s = -0.7902$; $N = 12$; $p < 0.01$).

The flies visited the inflorescences throughout the day but had an activity peak from 14:00 to 16:00 h and a smaller one from 08:00 to 10:00 h (Fig. 3b). Nectar was the main resource searched for by these visitors.

Flies from the family Calliphoridae were observed on *M. stipularis* during the whole day, being more frequent between 14:00 and 16:00 h (Fig. 3b). Many individuals of *Phaenicia eximia* were observed on the flowers of *M. stipularis* (Fig. 1d). Visits from individuals of *Chrysomia putoria* and *C. megacephala* were occasional and rare from *Hemilucilia segmentaria*. All Calliphoridae flies had a similar visiting behavior: they landed on the inflorescence and walked around searching for flowers. While removing nectar, the legs of the flies would touch the flower being visited and on neighbouring ones. To remove the nectar, the flies moved their tongue around the pistil, contacting the receptacle several times. When they visited flowers in the male phase, the tongue would touch the anthers and pollen grains would adhere to it (Figs. 1d, 4). When flowers in the female phase were visited, the tongue with pollen would contact the stigma. During the removal of nectar in flowers in the male phase, the oil droplets accumulated on the external wall of the anthers would adhere to the tongue of the flies. During this activity, the tongue would probably press against the glandular tubercles. The flies avoided flowers with a reddish colored nectariferous disk.

Species of Syrphidae were also found on the flowers throughout the day, presenting a visiting peak from 14:00 to 17:30 h (Fig. 3b). This family was represented by several species, where *Palpada* sp.1, *P. furcata* and especially *Ornidia obesa* were the most frequent (Table 3), and presented a visiting behavior similar to the one made by species of Calliphoridae. Some individuals of *Ornidia obesa*, *Palpada* sp.1, *Palpada* sp.2, *P. furcata* and *Ocyrtamus* sp. were sometimes observed removing pollen.

Flies of the families Sarcophagidae, Tachinidae, Muscidae, Stratiomidae and Tabanidae were classified as eventual or rare visitors (the

last two treated as "others" in Fig. 3b). All flies of these families presented a visiting behavior similar to the Calliphoridae flies. The family Lauxaniidae did not contact the reproductive organs of the flower while removing nectar due to the small size of their individuals.

According to the behavior and frequency of the visit, *Palpada* sp.1, *P. furcata* (Syrphidae) and especially *Phaenicia eximia* (Calliphoridae) and *Ornidia obesa* (Syrphidae) were considered to be the main pollinators of *M. stipularis* (frequent visitors, Table 3). Species with an adequate behavior but which visited the flowers only eventually (eventual in Table 3) were classified as secondary eventual pollinators and those with rare visits to the flowers were considered to be secondary rare pollinators (rare in Table 3). Flies from the family Lauxaniidae were classified as nectar robbers, removing nectar without touching the reproductive organs of the flower.

Few individuals of the three families of Hymenoptera visited the flowers of *M. stipularis* throughout the day (Fig. 3b, Table 3). The Hymenoptera were ranked as secondary pollinators, eventual or rare according to the frequency of the visits to the flowers (v. Table 3). They were not considered efficient pollinators since the style was damaged after some wasps' visits.

Discussion

Phenology. *Metrodorea nigra* and *M. stipularis* flower for a relatively short period (about six to seven weeks), and exhibit an annual flowering pattern most common among tree species of semideciduous forests in Southeastern Brazil and in other tropical seasonal forests (Morellato 1991). The populations of *M. nigra* and *M. stipularis* studied show synchronized flowering, that is, all the individuals of each species flower at the same time. Additionally, each of the species exhibits a synchronized peak in the number of flowers in the male and female phase. These are important characteristics, since the species of *Metrodorea* in this

study are self-incompatible and cross-pollination is essential for the fruit formation. Plants that flower synchronously may benefit from cross-pollination, since the pollinators transport the pollen from one plant to another (Rathcke and Lacey 1985, Taylor and Inouye 1985).

Dichogamy and breeding systems. Both species of *Metrodorea* studied are dichogamous and exhibit protandry. According to Lloyd and Webb (1986) definitions, the species of *Metrodorea* studied present an intrafloral, asynchronous dichogamy (each plant or inflorescence possesses flowers both in the female and male phases), and the individual flower exhibits complete protandry (there is no overlap between the presentation of pollen and receptivity of the stigma). This type of protandry is similar to the one described for other species of Rutaceae by Bertin and Newman (1993).

M. nigra and *M. stipularis* are self-incompatible, presenting an allogamous reproductive system associated to dichogamy. Although dichogamy has been interpreted as a mechanism for reducing self-fertilization (Proctor et al. 1996, Cruden 1988), several species display dichogamy associated with self-incompatibility (Lloyd and Webb 1986, Bertin 1993). We suggest that for the two species of *Metrodorea* studied here, self-incompatibility functions to avoid self-fertilization and dichogamy functions to avoid the interference between pollen and stigma and to grant a favourable position of the anthers in the male and female phases, favouring respectively, the removal and reception of pollen by the agents, as proposed by Lloyd and Yates (1982), Lloyd and Webb (1986), Bernhardt and Thien (1987). Webb (1985) and Imbert and Richards (1993) also studied species that exhibited protandry and strong self-incompatibility, suggesting the most probable function of dichogamy in these species is to avoid the interference between pollen and stigma.

Both species of *Metrodorea* produced many flowers and few fruits during the study period. Plants with hermaphrodite flowers and an allogamous reproductive system generally pre-

sent a low fruit production relative to the number of flowers produced (Stephenson 1981; Sutherland and Delph 1984; Sutherland 1986a,b; Sutherland 1987). We believe that the over-production of flowers is important to the attraction of pollinators, especially for *M. stipularis*. Many flowers may be acting only as pollen donors, resulting in a low fruit production in both species of *Metrodorea* studied, since there was a high density of pollinators with frequent visits and fruit abortion did not occur. Flowers in "excess" would act as males, donating pollen but not forming fruits (Bawa and Webb 1984; Sutherland and Delph 1984; Sutherland 1986a,b; Sutherland 1987) and would have an advantage enhancing the attractiveness of the plant to the pollinators (Augspurger 1980).

Pollination and floral biology. Although *Metrodorea nigra* and *M. stipularis* are sympatric, display a very similar floral morphology, are protandrous, have overlapping flower periods, and are both pollinated by flies, they do not share the same pollen vectors. The chief agents responsible for the pollination of *M. nigra* flowers are the flies *Pseudoptiloleps nigripoda* (Muscidae) and *Fannia* sp. (Fanniidae), and for *M. stipularis* *Phaenicia eximia* (Calliphoridae), *Palpada* sp.1, *P. furcata* and *Ornidia obesa* (Syrphidae). Differences in the odor (disagreeable in *M. nigra* and sweet in *M. stipularis*) as well as in the color of the flowers (brownish violet vs. cream) are probably determining the type and number of floral visitors in these species. Kluger (1950 apud Proctor et al. 1996) indicates that flies prefer brown-purple to yellow flowers when associated with excremental scents and the opposite in the presence of sweet scents. Therefore, for both species in this study the flower odor was probably responsible for the attraction of flies over a long distance. Several studies report that the fragrance and coloration of the flowers are responsible for attraction over a long distance and/or orientation of the visitors at a short distance (Proctor et al. 1996, Faegri and Van Der Pijl 1979, Vogel 1983, Knudsen and Tollsten 1991). According to Percival

(1965), flowers visited by flies present the odor as the most important attribute for attraction over a long distance.

The pollen grains of *M. nigra* and *M. stipularis* are transported on the tongue of the pollinators. The presence of "pollenkitt" and lipid substances derived from the external wall of the anthers and from the glandular tubercles, are important for making the pollen adhere to the visitors. Generally, species with auxiliary mechanisms for pollen adherence increase the efficiency of the pollination, when compared to species without such mechanisms (Cruden and Jensen 1979, Endress 1994). According to Faegri and Van Der Pijl (1979), the pollenkitt helps the pollen to adhere to smooth surfaces of pollinators with few hairs. The deposition of pollen on a restricted area of the body of the visitor (tongue), the presence of sticky lipid substances on the pollen grains as well as the high viability of the pollen optimize the efficiency of the fly pollination in *M. nigra* and *M. stipularis*.

The mean sugar concentration in the nectar of the flowers of *M. nigra* and *M. stipularis* was low, an uncommon characteristic in flowers pollinated by flies. According to Vogel (1983), flies and bees prefer flowers with high concentration of sugars. However, Percival (1965) cites a concentration of sugars varying from 5–15% for *Primula vulgaris* (Primulaceae), a species also pollinated by flies. In *Dendropanax cuneatum* (Araliaceae), another myiophilous species, there is only about 2% of sugars in the nectar (Pombal and Morellato 1995). Thus, there may be a great variation in the concentration of sugars in the flowers visited by flies (v. Corbet et al. 1979).

The fly pollination in *Metrodorea nigra* vs. *M. stipularis*. The disagreeable odor and dark, opaque color of *M. nigra* are characteristic of sapromyophily (e.g. Faegri and Van Der Pijl 1979, Endress 1994). The brown and brownish-purple colors are recognized to attract flies which feed on or breed in carrion and excrement, and flowers where these colors are found chiefly deceive or trap the flies (Proctor et al. 1996). However, *M. nigra* effectively offers a

reward for its fly visitors (nectar) and presents some other specific adaptations, besides color and odor, such as the "pollenkitt" and the lipid substances which help to make the fly pollination more effective. The time of anthesis (afternoon) and odor emission (early morning and late afternoon), associated with the dark color and disagreeable odor seem to have restricted the spectrum of floral visitors to a few species of flies; the enlarged corolla size, if compared with the other *Metrodorea* species (Kaastra 1982), appears to be an additional adaptation to make *M. nigra* flowers more attractive to Muscidae and Fanniidae fly species. A possible tendency of *M. nigra* towards sapromyophily has not been considered since we did not observe flies laying eggs and/or non-nectar flowers. The cream-color flowers of *M. stipularis* would explain why this species receives a larger diversity of visitors when compared to the opaque and dark *M. nigra* flowers. According to Silberbauer-Gottsberger and Gottsberger (1988) the white, yellow or cream color of flowers favours the attraction of a large variety of insects.

M. nigra could be considered as presenting a more specialized pollination system than *M. stipularis* if we assume a reduced number of flower visitors and effective pollinators as a tendency towards a more specialized (or less generalized) pollination system. The principal adaptations leading to a reduction on flower visitors and pollinator species presented by *M. nigra* were the changes on color and odor. The possible selective forces favouring that would be interference and pollinator-competition avoidance since *M. nigra* and *M. stipularis* are synchronopatric: they occur sympatric in the same vegetation type (semideciduous forests) and reproduce at the same time of the year.

According to Bradshaw et al. (1995), differences in floral traits such as color, shape and size are sufficient to ensure reproductive isolation. This is also valid for the two species of *Metrodorea*. Additional characteristics help to make the isolation more effective: the different patterns of odor emission (two times/day vs.

all day for *M. nigra* and *M. stipularis*, respectively), the large corolla size and reduced number of flowers per inflorescence (*M. nigra*), and the different habitat (understory: *M. nigra* and canopy: *M. stipularis*). But is the more specialized (or less generalized) pollination system of *M. nigra* more efficient? The control treatment performed on the same trees tested for breeding system showed a higher percent of fruit set for *M. stipularis* (36%) than for *M. nigra* (26%, Table 1). However, the overall flower/fruit counting performed in order to quantify the species phenology revealed a much smaller fruit set for *M. stipularis* which exhibited a mean of 1190.5 flowers produced and 5 fruits formed during the entire flowering season, or 0.4% fruit set. In contrast, *M. nigra* showed a mean flower production of 67.4 flowers/inflorescence and 1.1 fruit, or 1.6% fruit success, four times greater than *M. stipularis*. Therefore, the changes in color and odor presented by *M. nigra* has led to a more specialized and efficient pollination system.

Metrodorea nigra is the only species in the genus with dark purple flowers; all other species present yellowish-white, fragrant flowers with small corolla size arranged in huge inflorescences (Kaastra 1982). Its distribution overlaps with all *Metrodorea* species but *M. flavida*, which is restricted to Amazonian region. The distribution of *M. nigra* is concentrated in southeastern Brazil (São Paulo State), where it highly overlaps with that of *M. stipularis* (see map on Kaastra 1982: 110, fig. 35b). Thus, *M. nigra* seems to have been derived from the basic floral type described above and then spread out over a large geographic range along the southeast-northeast Brazilian coast side, but this assertions remain to be tested.

Fly pollination on tropical seasonal forests.

Among the pollination systems of tropical forests, fly pollination is considered to be irregular and uncertain, since these insects do not feed their young and use a variety of food resources (Faegri and Van Der Pijl 1979). However, many species of Diptera visit flowers and this order of insects is important for plant

pollination, especially in temperate ecosystems (Proctor et al. 1996). According to Endress (1994) flies are the second most important insect group of pollinators, after Hymenoptera, although the majority of them are not dependent on flowers for food and may be archaic pollinators. Despite its importance, fly pollination has rarely been recognized as a category in studies at the community level in tropical forests (e.g. Bawa et al. 1985, Kress and Beach 1994). The production of many small, generally light-colored flowers, with easy access to the nectar, usually attracting a large variety of visitors, especially favouring those with short buccal parts (Diptera and Hymenoptera), have been related to pollination by a variety of insects (Bawa et al. 1985; Kress and Beach 1994; Morellato 1991, 1995). However, careful studies have shown that these plants, considered generalists, may present more specific pollination modes (Bullock 1994, Pombal and Morellato 1995, pers. obs.). As observed for *M. nigra* and *M. stipularis*, detailed studies involving systematic observations, carried on several species from the semideciduous forest (at the SGR) initially considered to be pollinated by diverse insects, have reported flies as main pollinators (Morellato 1991, Passos 1995, Pombal and Morellato 1995, Figueiredo 1997, pers. obs.), stressing the importance of fly pollination on seasonal tropical forests. Recently, Waser et al. (1996) have pointed out that the occurrence of generalization and opportunism among pollination systems has been underestimated because several studies are biased by ignoring "improper" visitors. The present study and the above cited undertaken at SGR have demonstrated that just a small number of floral visitors are effective pollinators, revealing an opposite tendency towards a reduction of effective pollen vectors and "less generalized" pollination systems.

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