UNIVERSIDADE ESTADUAL DE CAMPINAS

BIOLOGIA REPRODUTIVA DE RUBIÁCEAS ARBUSTIVAS DE MATA ATLÂNTICA

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Dedico este trabalho às minhas avós
Maria, católica fervorosa
e Aristida (*in memorian*), líder operária.

“Nasceu no meu jardim
um mato que dá flor amarela.
Todo dia vou assistir à insetaria na festa.
Tem zoado de todo jeito:
tem do grosso, do fino, de aprendiz e de mestre.
É pata, é asa, é boca, é bico
É poeira e pólen na fogueira do sol.
Parece que a arvorinha conversa.”

Adélia Prado

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"I do not think anything in my scientific life has given me so much satisfaction as making out the meaning of the structure of heterostyled flowers."

Charles Darwin, 1876

ÍNDICE

Resumo. A polinização e os sistemas de incompatibilidade determinam grande parte do fluxo gênico em populações de plantas, portanto dados a esse respeito podem contribuir para a compreensão da manutenção da diversidade em diferentes ambientes. A distilia é um mecanismo reprodutivo comumente observado na família Rubiaceae, sendo caracterizada pela presença de dois morfos florais reciprocamente hercogâmicos, auto- e intramorfo-incompatíveis, e em número equilibrado na população. A ausência de alguma dessas características em grupos comumente distílicos pode evoluir devido à baixa disponibilidade ou comportamento pouco especializado de polinizadores, sendo interpretada como um caso de distilia atípica, ou de uma nova estratégia reprodutiva derivada da distila. O presente estudo investigou a biologia de polinização e as relações morfométricas e de compatibilidade entre morfos florais de cinco espécies de Rubiaceae de Mata Atlântica no sudeste brasileiro: *Psychotria jasminoides*, *Psychotria birotula*, *P. mapourioides*, *P. pubigera*, *P. nuda* e *Chiococca alba*. Foram coletados dados a respeito da biologia floral e de polinização, realizados experimentos reprodutivos de todas as espécies, além de medições de órgãos florais e estimativas da razão entre morfos das espécies distílicas. Apesar de todas as espécies possuírem flores tubulares, pequenas, diurnas e de produzirem néctar (exceto *C. alba*), algumas diferem entre si quanto ao principal grupo de polinizadores. Três espécies são tipicamente distílicas, duas são distílicas atípicas e uma possui apenas hercogamia (*C. alba*). Os resultados indicam perdas da hercogamia recíproca e desvios na razão entre morfos. No entanto, as espécies dimórficas apresentaram a síndrome da distilia funcionando conforme o esperado em populações estáveis, o que provavelmente é resultado da eficiência dos polinizadores em transportar pólen entre morfos, associada ao sistema de incompatibilidade. *Chiococca alba* possui uma estratégia diferente das esperadas para sua tribo, mas com características de ambas, como a hercogamia, típica da distilia, e deiscência das anteras na fase de botão, similar às espécies com apresentação de pólen no estilete. Trata-se de uma espécie com baixa freqüência de visitas, autoincompatível, com baixa formação de frutos por autopolinização e apomixia, além de taxa de frutos por polinização natural relativamente alta, sugerindo que as poucas visitas associadas aos frutos formados por autopolinização e apomixia mantêm população.

Abstract. Pollination biology and incompatibility systems to a large extent control gene flow within natural populations, thus data on this respect may contribute for the comprehension of diversity maintenance within plant populations of different environments. Distyly is a reproductive system commonly observed in the family Rubiaceae, being characterized by the presence of two floral morphs, which are reciprocally herkogamous, self- and intramorph-incompatible, in a equivalent number of individuals within the population. The absence of any one of these features in typically distylous plants may evolve as a result of low availability or inefficient behavior of pollinators, being interpreted as a case of atypical distyly, or a new reproductive strategy derived from it. This study presents data on the pollination biology, compatibility relations of all species, and morphometric comparisons between floral morphs of five Rubiaceae species from the Atlantic rain forest, SE Brazil: *Psychotria jasminoides*, *P. birotula*, *P. mapourioides*, *P. pubigera*, *P. nuda* e *Chiococca alba*. Data were collected through observation of floral events, pollinators and reproductive experiments for all species, floral organs measurements and estimative of morph ratio for distylous ones. Aside presenting small, tubular, diurnal, nectariferous (except one of them) flowers, some of the species studied are pollinated by different group of pollinators. Three species are typically distylous (*P. jasminoides*, *P. birotula* e *P. mapourioides*), two are atypical ones (*P. pubigera* e *P. nuda*), and one is herkogamous (*C. alba*). Results indicate some deviations of distylous patterns, as the lost of reciprocal herkogamy or isoplethy. Nevertheless, dimorphic species present distylous syndrome working as expected in stable populations, which probably is a result of the efficiency of pollinators in perform intermorph pollen flow in addition with the self-incompatibility system. *Chiococca alba* has a reproductive strategy different to those expected for its tribe, but including features of both. Similarly to distylous plants, its flowers are herkogamous and, as those species with secondary pollen presentation on the style, pollen is released at the bud stage. This species presents a low frequency of visits, is self-incompatible, with low fruit production through self-pollination and apomixis; nevertheless, it has relatively high fruit set under natural conditions, which suggests that those visits, together with the fruits produced by self-pollination and apomixes, maintain the population at the study site.

INTRODUÇÃO GERAL

A família Rubiaceae é um grupo de plantas tipicamente lenhoso (Cronquist 1981) que constitui um dos mais importantes componentes do subosque das florestas neotropicais (Gentry e Emmons 1987, Laska 1997). Apesar de incluir diversas estratégias sexuais, a apresentação de pólen no estilete e a distilia são os mais importantes, e muitas vezes podem caracterizar as tribos da família (Robbrecht 1988), apesar de várias exceções serem encontradas (Bawa *et al.*1985, Imbert & Richards 1993, Richards & Koptur 1993, Castro e Oliveira 2001).

A distilia é um dimorfismo floral geneticamente relacionado a um sistema esporofítico de incompatibilidade, onde os morfos florais brevistilo e longistilo diferem principalmente quanto à altura dos órgãos sexuais, ao que denominamos hercogamia recíproca (Ganders 1979, Dulberger 1992). Esta estratégia reprodutiva é considerada um exemplo de convergências morfológica e fisiológica que desfavorece a autofecundação e maximiza as aptidões masculina e feminina (Lloyd e Webb 1992a, b). Várias famílias contêm gêneros distílicos, entretanto a grande maioria dos gêneros está incluída na família Rubiaceae, principalmente na tribo Psychotrieae, onde a distilia é considerada uma característica primitiva (Hamilton 1990, Barrett 1992).

A hercogamia recíproca associada à auto-incompatibilidade, à incompatibilidade intramorfo e à razão equilibrada entre os morfos na população, também chamada isopletia, são características típicas de espécies distílicas (Barrett 1992 e referências citadas). Por outro lado, a assimetria floral entre morfos como corola, anteras e lobos estigmáticos maiores em flores longistilas, além de diferenças no número, tamanho e escultura da exina dos grãos de pólen, e tamanho e morfologia das papilas estigmáticas constituem características secundárias freqüentemente, mas nem sempre, observadas (Dulberger 1992, Herman *et al.* 1999).

Apesar dos padrões morfológicos e de compatibilidade serem bem estabelecidos para plantas distílicas, existe uma variedade de modificações evolutivas desta síndrome. Plantas pertencentes a grupos comumente distílicos que apresentam tais desvios não são consideradas distílicas típicas, mas espécies onde uma nova estratégia reprodutiva evoluiu a partir da distilia (Hamilton 1990, Pailler e Thompson 1997). Um importante trabalho de referência, que constitui a mais recente compilação do histórico e das teorias que envolvem a evolução, manutenção e quebra da distilia é o de Barrett (1992).

Muitos dos estados derivados da distilia são observados em ambientes tropicais, onde existe forte ocorrência de espécies distílicas e uma grande diversidade de pressões seletivas interferindo na estabilidade das populações (Barrett e Richards 1990). Muitas vezes a depleção ou ausência de polinizadores eficientes está relacionada ao estabelecimento de tais estados derivados, uma vez que espécies distílicas dependem de vetores que, além de visitarem ambos os morfos florais, entrem em contacto com os dois níveis de órgãos sexuais para que haja formação de sementes. Um polinizador eficiente deve possuir, portanto, comportamento dinâmico, para transportar pólen entre os morfos, e aparelho bucal suficientemente longo, para contactar os órgãos do nível inferior adequadamente (Khon e Barrett 1992).

A depleção de polinizadores eficientes em habitats marginais à distribuição das espécies, por exemplo, pode influenciar a seleção de espécies homostílicas (i.é., possuem flores com órgãos sexuais de mesma altura) autocompatíveis, conforme sugerido por Charlesworth e Charlesworth (1979) e Sobrevilla *et al.* (1983). O comportamento ineficiente de polinizadores parece também estar intimamente relacionado ao surgimento de dioicia a partir de grupos distílicos. Animais de aparelho bucal curto entrariam em contato principalmente com os órgãos do nível mais alto, fazendo com que flores longistilas funcionassem apenas como femininas e as brevisitilas como masculinas, como sugerido por Beach e Bawa (1980), Khon e Barrett (1992) e Pailler *et al.* (1998).

Assim como na maioria das espécies distílicas, as flores tubulares das Rubiáceas favorecem visitantes com aparelho bucal longo, sendo mais comumente polinizadas por borboletas e abelhas com probóscide longa (Taylor 1996), e esporadicamente por beija-flores (Passos e Sazima 1995, Castro e Oliveira 2001) e mariposas (Imbert e Richards 1993). A flor tubular restringe a direção do aparelho bucal do visitante dentro da corola, fazendo com que constate os dois níveis de órgãos sexuais antes e depois de atingir o néctar, secretado na base da flor (Khon e Barrett 1992). Björkman (1995) mostrou que a distilia não é eficiente em aumentar a polinização cruzada em flores tipo prato, e que o recebimento e o despacho de pólen é mais eficiente no morfo longistilo.

A estreita relação entre morfologia floral, morfologia e comportamento de polinizadores e sucesso reprodutivo de espécies distílicas levou vários autores a investigar a influência de tais variáveis no fluxo de pólen daquelas plantas. A maioria dos estudos indicou que, assim como nas flores tipo aberto, a distilia é apenas parcialmente eficiente em promover transferência intermorfo de pólen, uma vez que grãos de pólen de flores longistilas são transportados mais eficientemente para estigmas de flores brevistilas do que o contrário. Apesar do morfo brevistilo receber menor quantidade de pólen, recebe freqüentemente maior proporção de grãos compatíveis (Barrett 1992, Stone 1995).

Stone (1996) comparou os padrões de transferência de pólen entre os vários polinizadores de *Psychotria suerrensis*, e observou que tanto abelhas Euglossine quanto borboletas transferem quantidades semelhantes de pólen. No entanto, as abelhas são os visitantes mais freqüentes e mais eficazes no transporte polínico intermorfo. Riveros *et al.* (1995) registrou baixa taxa de visitação associada à autocompatibilidade em *Hedyotis salzmannii*, e mostrou a existência de uma maior produção de sementes resultantes de cruzamentos intermorfo do que intramorfo. Em *Palicourea padifolia* (Ree 1997) a tendência à maior deposição de pólen compatível no morfo brevistilo não foi corroborada.

Além da homostilia e da dioicia, a monomorfia - presença de apenas um morfo na população - também pode constituir uma estratégia reprodutiva derivada da distilia, como registrado por Hamilton (1990) para várias espécies de *Psychotria*. Desvios mais sutis também podem ser encontrados, como a ausência de hercogamia recíproca, que na maioria das vezes se dá pela similaridade na altura de anteras dos dois morfos florais (Sobrevilla *et al.*1983, Hamilton 1990).

Richards e Koptur (1993) registraram distilia morfológica em *Guettarda scabra*, mas também uma variação morfométrica incomum dentro dos morfos, associada à sobreposição nas alturas de estigma e antera entre ambos. Uma investigação da ontogenia floral mostrou uma divergência, entre os morfos florais, nas taxas de crescimento de estiletes durante os últimos estágios do desenvolvimento da flor, o que corrobora a teoria de que a distila evoluiu em um ancestral semelhante ao morfo longistilo, e que o morfo brevistilo surgiu a partir de uma mutação que resultou na limitação do crescimento do estile.

Apesar da reação de incompatibilidade ocorrer mais comumente no estigma, grupos tipicamente distílicos podem conter espécies com diferentes graus nas reações de incompatibilidade, como o crescimento de tubos incompatíveis até a metade ou à base do estilete, como observado por Richards e Koptur (1993). Sobrevilla *et al.*(1983), investigando populações simpátricas de *Palicourea fendleri* e *P. petiolaris*, observou que a segunda espécie apresentava autocompatibilidade e um maior número de indivíduos longistilos em algumas populações, possivelmente resultantes de mutações relacionadas ao comportamento do pólen de flores longistilas.

Muito poucos trabalhos investigam a síndrome da distilia em grupos proximamente relacionados, como Bawa e Beach (1983) e Hamilton (1990) realizaram para Rubiaceae e *Psychotria* subgênero *Psychotria*, respectivamente. Ainda mais escassos são os trabalhos relacionados a espécies de Rubiáceas distílcas de Mata Atlântica, como o de Passos e Sazima (1995) envolvendo uma espécie herbácea, *Manettia luteo-rubra*, e o de Machado e Loyola (2000), com *Borreria alata* (Rubiaceae). A Mata Atlântica é uma das formações com maior diversidade do planeta, cuja distribuição encontra-se fortemente limitada pela ação antrópica (Viana e Tabanez 1997), e onde esta família constitui um dos grupos mais importantes do subosque (Gentry e Emmons 1987, Laska 1997).

Os objetivos do presente estudo são:

- investigar a biologia de polinização das seguintes Rubiáceas arbustivas, encontradas em uma área de Mata Atlântica no sudeste brasileiro: *Psychotria jasminoides*, *P. birotula*, *P. mapourioides*, *P. pubigera*, *P. nuda* e *Chiococca alba*;
- investigar as relações morfométricas e de compatibilidade entre os morfos de espécies dimórficas, relacionar esses dados com a razão de morfos nas populações e determinar se as mesmas são tipicamente distílicas.

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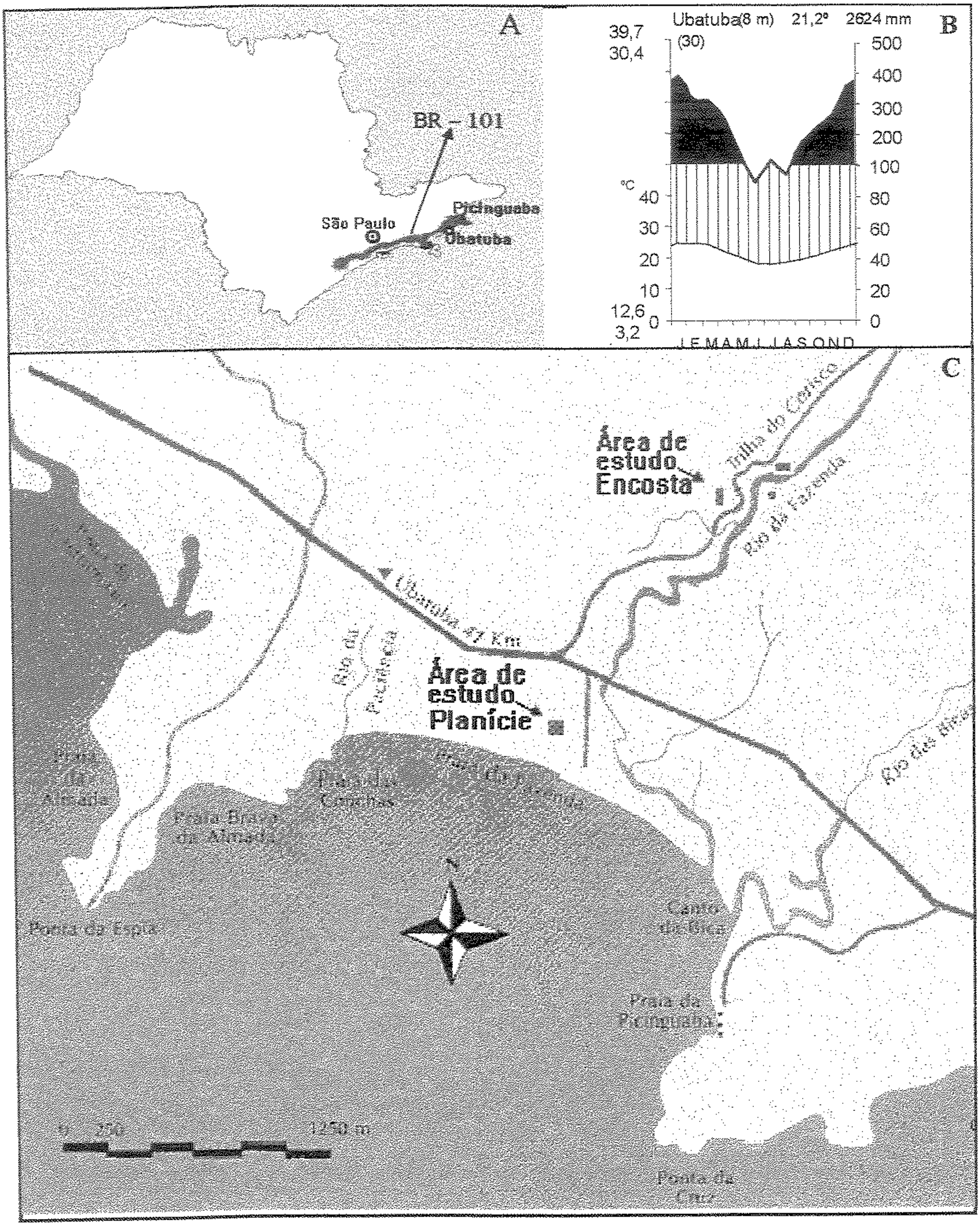

Figura 1. (A) Localização do Núcleo Picinguaba no Parque Estadual da Serra do Mar SP. (B) Diagrama climático de Ubatuba, SP para o período de 1961 a 1990. (C) Localização da área de estudo na planície e encosta do Núcleo Picinguaba.

CAPÍTULO I

Biologia da polinização de Rubiáceas distílicas de Mata Atlântica

Pollination biology of distylous Rubiaceae from Atlantic rain forest, SE Brazil[§]

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Running-title: Pollination of distylous Rubiaceae from Atlantic rain forest.

Key words: Atlantic rain forest, bees, butterflies, distyly, hummingbirds, pollination, *Psychotria*, Rubiaceae.

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Abstract. Data on pollination biology constitute important clues for the comprehension of pollen flow and genetic differentiation in plant populations. Pollinator type, availability and behavior may modify morphological and mating patterns in populations of typically distylous species. This study investigates the pollination biology of five distylous species of Rubiaceae in the Atlantic rain forest, SE Brazil. Data on flowering phenology, floral lifespan, stigmatic receptivity, pollen availability, nectar volume and concentration, and pollinator activity were collected. The species studied flower sequentially throughout the wet season, and produce terminal inflorescences, which bear small, tubular, diurnal, nectariferous (one exception) flowers. Despite these similarities, some of the species studied are pollinated by different groups of pollinators, probably due to their distribution, availability of flowers and corolla length. On the other hand, pollinator specificity does not seem to be so important for distylous species. Long mouthparts, like those of most of the recorded pollinators, may reach lower sexual organs and be sufficient to perform legitimate pollination and maintain levels of intermorph mating. Besides some restrictions on pollen flow, related to pollinator behaviour, the species studied seem to present seed-set that maintain their populations at the study area. This result may be probably achieved through the interaction between pollination biology and the self- and intramorph-incompatibility observed.

Introduction

Data on pollination biology constitute important clues for the comprehension of pollen flow and genetic differentiation in plant populations (Lloyd 1979[23], Proctor et al. 1996[30]). Low availability and/or efficiency of pollinators, for example, are considered selective pressures that probably contributed to the evolution of distyly in Angiosperms (Charlesworth and Charlesworth 1979[5], Ganders 1979[13], Barrett 1992[1]). Pollinator availability and behavior may also modify morphological and mating patterns in populations of typically distylous species, leading to the evolution of self-compatibility (Sobrevilla et al. 1983[30], Barrett 1992[1], Richards and Koptur 1993[32], Ree 1997[27]), dioecy (Beach and Bawa 1980[2], Pailler et al. 1998[28]), fruit set biased toward one morph (Björkman 1995[3]) or even the loss of reciprocal herkogamy (Hamilton 1990[15], Dulberger 1992[9]).

Reciprocal herkogamy is one of the most conspicuous features of distylous species, and consists of reciprocal differences in anther and stigma heights of two floral morphs. In addition to self- and intramorph-incompatibilities, this morphological trait maximizes outcrossing rates of distylous populations, which are generally isopletic, *e.g.*, have equivalent number of thrum and pin individuals (Ganders 1979[13], Dulberger 1992[9]). Several theories try to explain the adaptive significance of distyly, and almost all agree with the Darwinian "pollinator theory": reciprocally hekogamous flowers achieve intermorph-pollination, *e.g.*, pollinators contact same-level floral organs with the same region of their body (Ganders 1979[13], Dulberger 1992[9]).

Most distylous genera are included in the family Rubiaceae (Robbrecht 1988[33], Barrett 1992[1]), which forms an important component of the understory layer in Neotropical forests (Gentry and Emmons 1987[14], Laska 1997[22]), like the Atlantic forest. Despite this, distyly has been poorly studied in this environment (but see Passos and Sazima 1995[29]), which attains one of the highest levels of biological diversity in the world, although being currently restricted to five percent of its original distribution (Viana and Tabanez 1997[38]).

The objectives of this study were: 1) to investigate floral biology of four distylous Rubiaceae species in the Atlantic rain forest, SE Brazil; 2) to observe floral visitors and pollinator species, frequency of visits and behavior, comparing these data between floral morphs; 3) to relate these data with available information on the breeding system of these plant species.

Material and Methods

Study site and species studied - The study was conducted in the "Parque Estadual da Serra do Mar, Núcleo Picinguaba", SE Brazilian coast (44°48' - 44°52W and 23°20' - 23°22'S), between October 1998 and May 2000 (Fig. 1A, p.8). The climate of Serra do Mar, where the study site is located, is tropical wet (*sensu* Walsh 1996[40]) or tropical rainy (*sensu* Köppen 1948[19]) with rain occurring every month (Fig. 1B, p.8). The species studied are the shrubs *Psychotria jasminoides* (Cham.) Müll. Arg., *Psychotria birotula* L. B. Sm. and Downs, *P. mapourioides* DC. and *P. pubigera* Schltdl. Data on *P. nuda* (Cham. and Schltdl.) Wawra, collected by Castro and Araujo (unpub. data) in 1994, in the same area, will be used here for comparison.

The Atlantic rain forest includes coastal plain and slope areas, which represent distinct environments (Viana and Tabanez 1997[38]). *Psychotria jasminoides*, *P. birotula* and *P. pubigera* occur mainly in the slope area, where the studied populations were located (Fig. 1C, p.8). *Psychotria mapourioides* distribution is restricted to the coastal plain area, and the population studied is located along ca. 300m (Fig. 1C, p.8). *Psychotria nuda* is found in both the coastal plain and slope areas, and the study population is distributed in the same area of *P. mapourioides*. Most individuals of *P. jasminoides*, *P. birotula*, *P. mapourioides* and *P. nuda* seem to be more clumped than those of *P. pubigera*.

Psychotria jasminoides, *P. birotula* and *P. mapourioides* are typically distylous, *e.g.* present reciprocal herkogamy, self- and intramorph-incompatibility and isopletic populations (Castro and Oliveira, unpublished data). *Psychotria nuda* does not have exactly reciprocal herkogamy, and the population of *P. pubigera* is not isopletic, but both species have the typical distylous incompatibility system (Castro and Oliveira, unpublished data).

Flowering phenology - The flowering phenology of the slope species had previously been studied by Gajardo (1997[11]) using the Fournier (1974[11]) method and will be used here for comparison. Data were collected for *P. mapourioides* using the same method of Gajardo's study, for a similar period of time (one year), for 20 individuals/morph/species.

Floral biology – The anthesis period was followed through daily monitoring of ten tagged flowers/species/morph, from at least three individuals/species. Stigma receptivity was tested by the peroxidase technique (Kearns and Inouye 1993[18]) on flowers from different individuals of both morphs, three times a day: early morning, midday, and afternoon.

Data on corolla length and diameter were collected elsewhere (Castro and Oliveira, unpublished data) and compared here using the ANOVA analysis, and the Tukey HSD Multiple Comparisons (Sokal and Rohlf 1981).

Nectar concentration and volume were collected only for *P. mapourioides* and *P. pubigera*. Flowers are too small and produce insufficient volume of nectar for direct use of a refractometer, so nectar was collected in ca. 30 bagged flowers/morph, from several individuals, between 10:00 and 11:00 h, when it was possible to extract the minimum volume necessary for the reading on the refractometer (Dafni 1992[7]). The value shown by the refractometer was considered as sucrose equivalents. For volume measurements the same method (accumulative extraction in capillaries) was used and spotted on a no. 1 Whatman filter paper. The spot diameter was measured and compared with spots of known volume made in the laboratory with solutions of similar concentration to the floral nectar (modified from Des Granges 1978[9]). Nectar measurements of the other species were not precise, and we decided to exclude those data.

The presence of osmophores was tested with a neutral red solution using a minimum of 10 intact, fresh flowers of distinct individuals from each morph of all species (Dafni 1992[7]). Volatile compounds were extracted with $CHCl_2$. The dicloromethanic layer was filtered through anhydrous $MgSO_4$ then gently reduced on N2 stream to 100 ml. To identify each compound, a GC-MS analysis was performed in a GC system (HP6890) coupled with a MS detector (HP5973) equipped with a fused silica capillary column (HP5MS, 30m x 0.25 mm x 0.25 mm, Whitten et al. 1993[44]).

Floral visitors - Floral visitors were determined by focal observation from 6:00 to 16:00 h in both floral morphs of all species. Total periods of observation of each species were 10 h for *R jasminoides*, *P. birotula* and *P. mapourioides* and 15 hours for *P. pubigera*. During focal observations, the floral visitors' species morph, the number of visits and behavior on the flower, as contact with stigma and anthers, kind of reward collected, collecting behavior and interaction with other visitors were recorded. Insect visitors were collected and identified as far as possible.

Voucher specimens of the plants are deposited in the Universidade Estadual de Campinas herbarium (UEC 118309-118315).

Results

Flowering phenology and floral biology - Flowering peaks of the species studied are almost sequential along the wet season (Fig. 2), when individuals produce terminal inflorescences bearing small, tubular flowers, which open early in the morning and wilt at dusk. Anthesis of both morphs of all species is synchronic within and between individuals. Flowers are all sympetalous, synsepalous, epigenous and apparently reciprocally herkogamous, with a basal nectariferous disc inside the corolla. Androecium is isostemon with epipetalous stamens; gynoecium is syncarpic, and consists in a two-loculated, two-ovulated ovary, and a bilobated, non-sensitive stigma.

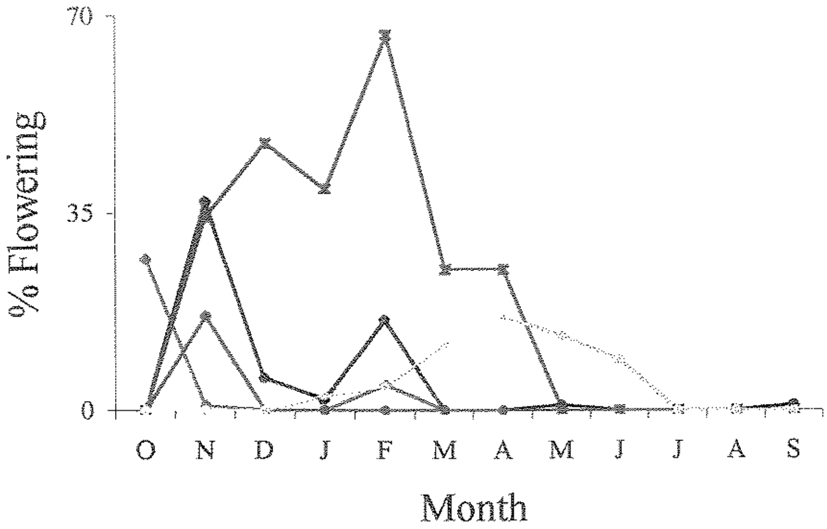

Figure 2. Fournier percentage of flowering individuals of Psychotria species in the Atlantic rain forest, SE Brazil. Data for *Psychotria jasminoides* (blue), *Psychotria birotula* (red), *P. pubigera* (pink) and *P. nuda* (yellow) are from Gajardo (1997[]), and those for *P. mapourioides* (black) were collected in 1998.

Corollas are white/cream (Fig. 3D-F), except in *P. pubigera* and *P. nuda*, which are pale (Fig. 3A-B) and bright yellow, respectively. *Psychotria jasminoides* have the largest corolla length, being followed by *P. mapourioides* and *P. pubigera*, which corolla sizes are similar, *P. nuda* and *P. birotula* ($P<0.01$ and $n=30$ flowers/morph/species for all, Fig.4). Corolla diameter is less variable than corolla length within species studied: *P. nuda* presents the largest diameter, being followed by *P. mapourioides* and *P. jasminoides, P. pubigera* and *P. birotula*, which present similar diameters ($P<0.01$ for all, Fig. 4).

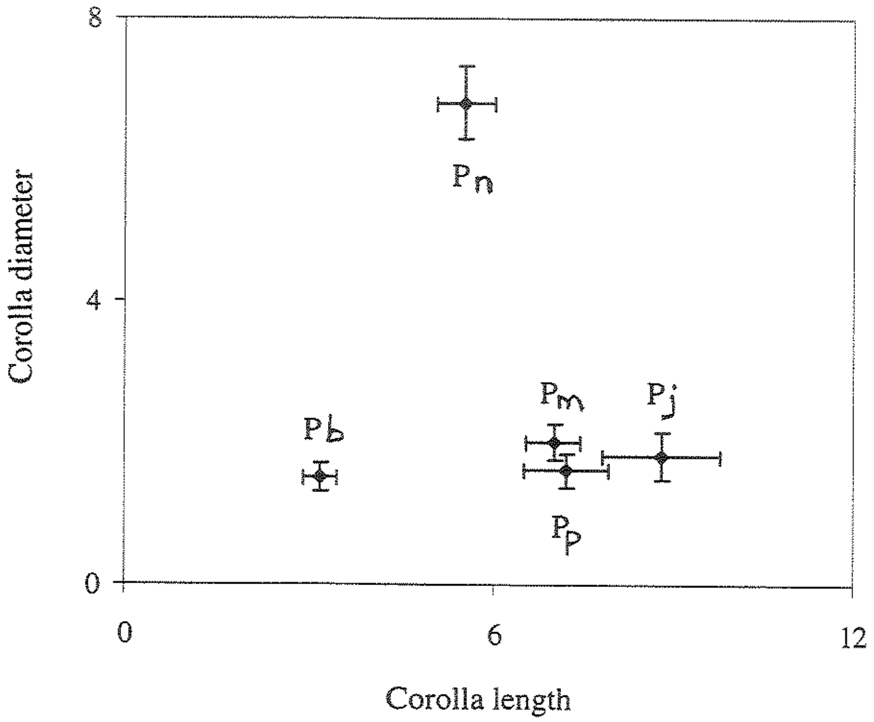

Figure 4. Corolla length and diameter (mm) of *Psychotria* species in the Atlantic rain forest, SE Brazil. Pj: *Psychotria jasminoides*, Pb: *P. birotula*, Pm: *P. mapourioides*, Pp: *P. pubigera*, Pn: *P. nuda*. Bars: standard deviations

Pollen and nectar are available, and the stigma is receptive, during all of the floral lifespan. In some species, such as *P. birotula*, nectar secretion starts about one and two hours after the beginning of the anthesis. Although both floral morphs produce similar volumes of nectar, it is more concentrated in thrum flowers (Table 2).

Neutral red stained the corolla lobes positively, suggesting that petals might act as osmophores (osmophores were not investigated for *P. nuda*). However, no species presented perceptible floral odor, and no volatile compound was isolated from dichloromethane extraction.

Table 2. Comparison of nectar volume (µl) and concentration (sucrose equivalents) between floral morphs of distylous *Psychotria* species in the Atlantic rain forest, SE Brazil. (n): number of flowers.

	Volume			Concentration		
Species	Thrum (n)	Pin (n)	P	Thrum(n)	Pin(n)	P
P. mapourioides	0.27 (8)	0.30 (9)	>0.05	5.23 (18)	2.57 (18)	<0.01
P. pubigera	0.39 (10)	0.36 (10)	>0.05	10.83 (15)	4.28 (39)	<0.01

Floral visitors. We were unable to distinguish, during focal observations, between species of the same genus in *Parides* and in *Malinaea*, nor between the following pairs of bee species: *Augochlora* sp. ♂ / *Augochloropsis* sp. ♀, Tapinotaspidini ♀ / Augochlorini ♀, *Trigona spinipes* / *Partamona helleri*. Thus data of these visitors were grouped both in Table 4 and in statistical analysis.

Floral visits to the species studied occurred throughout the day, especially from 9:00 to 14:00 h; *Psychotria mapourioides* was the only species visited early in the morning (as early as 7:00 h). Pin flowers of *P. jasminoides*, *P. birotula* and *P. pubigera* were more frequently visited than thrum ones, whereas floral morphs of *P. mapourioides* received similar number of visits (Table 3).

Most visitors of each plant species explored both floral morphs: 84% in *P. jasminoides*, 71.5% in *P. birotula* and 100% in *P. mapourioides*. Moreover, 43.7% of the visitors that explored both floral morphs showed preference for one floral morph (mainly pin ones, Table 4). In *Psychotria pubigera* only 55% of the visitors that explored both floral morphs preferred one of them (mainly thrum ones, Table 4). Thus, floral visitors may be divided into two groups: one that visits both floral morphs of each species, and therefore may perform legitimate (intermorph) crosses, and the other visits only one morph. Within the first group there are species that constitute the main group of pollinators of each species, especially because of their relatively high frequency of visits.

Table 3. Number of legitimate visits on thrum and pin flowers of dimorphic *Psychotria* species in the Atlantic rain forest, SE Brazil. Asterisk indicates statistically different values.

Plant species	Thrum	Pin	χ^2
P. jasminoides	976	773	23.56*
P. birotula	74	244	45.44*
P. mapourioides	162	146	0.83
P. pubigera	102	166	15.28*

Psychotria jasminoides is visited mainly by butterflies, and *Parides* spp. (Table 4, Fig. 3C-D) are its most important pollinators. The visits of butterflies in general occurred especially in the afternoon. More than one individual of *Parides* spp. was frequently observed visiting the same plant, even the same inflorescence. They landed on the inflorescence and visited several flowers each one, generally spending more than five minutes per plant. They were frequently observed visiting twice the same flower, and many times visiting sequentially the two floral morphs. Individuals of *Parides* spp. and *Malinaea* spp were sporadically observed visiting legitimately flowers of *P. mapourioides* and. in *P. nuda*, respectively.

Table 4. Floral visitors and their relative frequency of legitimate visits on flowers of *Psychotria* species in the Atlantic rain forest, SE Brazil. Pj: *Psychotria jasminoides*, Pm: *P. mapourioides*, Pb: *Psychotria birotula*, Pp: *P. pubigera*, Pn: *P. nuda*, T: thrum flowers, P: pin flowers, T + P: thrum + pin flowers, X: Visits recorded out of focal observation sessions. *: floral morph more frequently visited (P<0.01).

Species group**	Pj			Pb			Pm			Pp			Pn
	T	P	T+P	T	P	T+P	T	P	T+P	T	P	T+P	T+P
Hymenoptera													
Augochlora sp. ♂ / *Augochloropsis* sp. ♀	-	-	-	8.1	8.2*	8.2	-	-	-	4.6	-	1.7	-
Ceratinula cf mulleri	-	-	-	37.8	17.6	22.3	-	-	-	-	1.6	1	-
Melipona rufiventris	-	-	-	-	-	-	2.4	0.9	1.6	-	58.2	36.4	-
Paratetrapaedia cf lineata ♂	5.9	7.6	6.6	6.7	7.4*	7.2	-	-	-	29.4*	3.8	13.4	-
Tapinotaspidini ♀ / Augochlorini ♀	-	-	-	-	7.4	5.7	-	-	-	2.7	-	1	-
Trigona spinipes / *Partamona helleri*	38.7*	1.6	22.6	41.9	55.3*	52.2	92.3	92.2	92.3	36.7	26.9	30.6	X
Non-collected bees	-	1.1	0.5	5.4	3.7*	4.1	5.3	6.9	6.1	7.3	7.2	6.5	-
Lepidoptera													
Malinaea ludovica / *M. ludovica parayia*	0.4	0.5	0.4	-	-	-	-	-	-	-	-	-	X
Parides anchises nephalion/ *P. tros*	52.3	73.3	61.4	-	-	-	X	X	-	-	-	-	-
Non-collected Lepidoptera	2.7	15.8*	8.4	-	-	-	-	-	-	19.3	-	7.2	-
Other non-collected insects	-	-	-	-	0.4	0.3	-	-	-	2.2	-	2.1	-
Hummingbirds													
Phaethornis ruber	-	-	-	-	-	-	-	-	-	-	-	-	19.2
Ramphodon naevius	-	-	-	-	-	-	-	-	-	-	-	-	X
Thalurania glaucopis ♀	-	-	-	-	-	-	-	-	-	-	-	-	80.9
Thalurania glaucopis ♂	-	-	-	-	-	-	-	-	-	-	-	-	X

** Each group includes visitor species morphologically similar that were not distinguished by the authors during focal observ

Psychotria birotula, *P. mapourioides* and *P. pubigera* are visited mainly by the bees *Trigona spinipes* and *Partamona helleri* (Table 4, Fig. 3E-F). These bees may either pollinate (Fig. 3F) or pillage the flowers, the last through holes made at the base of the corolla tube or by collecting pollen directly of the anthers of thrum flowers

Trigona spinipes and *P. helleri* prefer to collect pollen early in the morning in thrum flowers of *P. mapourioides*, landing directly on the anthers, which had been recently opened and pollen was abundant (Fig. 3E). Pollen availability diminishes throughout the morning, and they start to collect nectar legitimately until afternoon, firstly checking the anthers for pollen, and introducing the head across the anthers (Fig. 3F), when both the head and the abdomen may be contaminated with pollen. During legitimate visits on pin flowers these bees contact the stigmatic surface with the same body regions that had touched thrum anthers. When these bees landed on an inflorescence, they explore almost all flowers from it (sometimes twice the same flower), walking from one flower to another, sporadically contacting pin stigmas with the abaxial region of the abdomen.

The visiting behavior of *T. spinipes* and *P. helleri* to the flowers of *P. birotula* and *P. pubigera* was similar to *P. mapourioides*. They contact thrum anthers and pin stigma with the abdomen during legitimate visits and frequently walk on the inflorescences.

Ceratinula cf. *mulleri* is an important pollinator of *P. birotula*, visiting legitimately both floral morphs and contacting thrum anthers and pin stigma with the abdomen. These small bees may also collect pollen directly from the anthers of thrum flowers, and try to collect nectar at the base of pin corollas.

The bee *Paratetrapaedia* cf. *lineata* seems to play a secondary role in the pollination of all but one species, *P. mapourioides* (Table 4). Its high frequency of visits in *P. pubigera* is due to a visiting section that occurred early in the morning, when this bee visited legitimately 16 flowers of a thrum individual. In thrum flowers of *P. pubigera* this bee may also collect pollen from the anthers; during their visits to *P. pubigera* flowers, more than one individual may share the same inflorescence, frequently touching the anthers of thrum flowers, and the stigma of pin ones, with the abdomen and/or with the posterior legs.

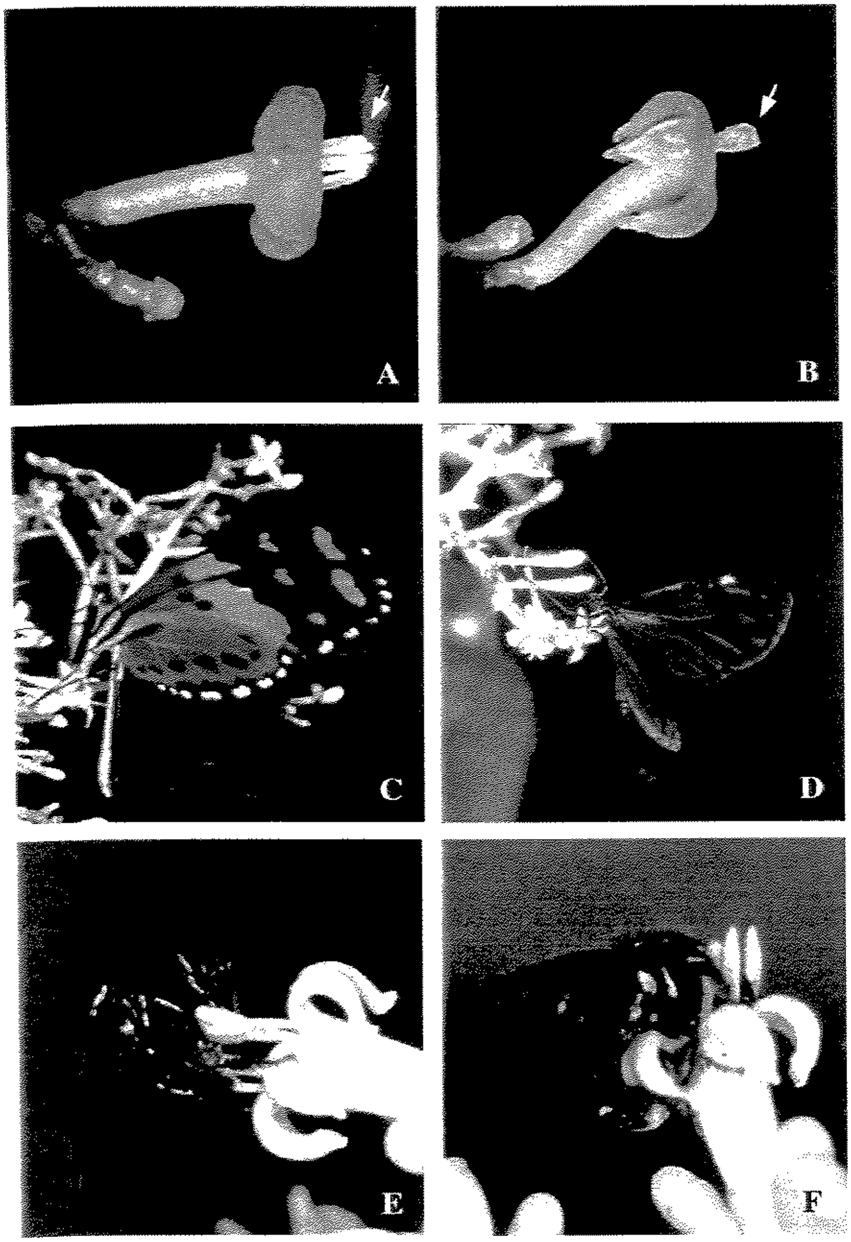

Figure 3. Flowers and floral visitors of *Psychotria* species in the Atlantic rain forest, SE Brazil. A, B. Thrum and pin morphs of *Psychotria pubigera*; note the exerted stamens and stigma, respectively (arrows). C, D. *Malinaea* sp.and *Oleria monarca* visiting flowers of *Rudgea jasminoides*. E, F. Bees collecting pollen and nectar respectively from thrum flowers of *P. mapourioides*. Bar: 2 mm.

Males of the bee *Augochlora* sp., females of *Augochloropsis* sp. and individuals of *Augochlora* sp contacted the anthers of thrum flowers and stigma of pin ones of *P. pubigera* with the head, and were observed robbing nectar at the base of thrum corollas. All the visits of *Melipona rufiventris* were legitimate, very rapid, and sometimes it may prevent the visits of *T. spinipes* and *P. helleri* to *P. pubigera* flowers. *Melipona rufiventris* was frequently observed visiting all the flowers of one inflorescence, both in *P. mapourioides* and *P. pubigera*.

Occasional floral visitors as non-identified bees, butterflies and moths were observed in all plant species (Table 4). The bees were the most frequently ones in all but one species, *P. jasminoides*, which occasional visitors were mainly Lepidoptera species. Eventual visitors may act as pollinators, or may visit only one floral morph, as species of Tapinotaspidini e Augochlorini ♀ in *P. birotula* and *P. pubigera*.

Psychotria nuda was almost exclusively pollinated by the hummingbirds *Ramphodon naevius* and *Thalurania glaucopis* (Table 4), which visited sequentially the flowers of *P. nuda* during its flowering period, and presented distinct foraging strategies, which may result in different effectiveness in promoting intermorph pollinations (Castro and Araujo, unpublished data).

We were not capable to observe the pollinators contacting lower sexual organs of none of the species studied.

Discussion

Floral traits of the species studied are clearly related to their floral visitors. Diurnal, pale-colored, tubular and small flowers, like those of *P. jasminoides*, *P. birotula*, *P. mapourioides* and *P. pubigera* are known to be more frequently pollinated by butterflies and bees, which visit flowers that present nectar and pollen as resources (Proctor et al. 1996[30]). On the other hand, contrasting colors, like those of *P. nuda* flowers, and a relatively larger corolla diameter generally attract hummingbirds (Proctor et al. 1996[30], but see Waser et al. 1996[39]). Like the species studied, the majority of Rubiaceae is known to be more commonly pollinated by butterflies and long-tongued bees (Taylor 1996[37]), although hummingbirds (Castro and Oliveira 2001[4]) and moths (Richards and Koptur 1993[32]) may also pollinate them.

The nectar volume recorded here is included within the range expected for melitophilous flowers (Opler 1983[27]), whereas concentration of sugars is somewhat lower to the generally recorded (Cruden et al. 1983[6]).

In addition to flower morphology, color and floral resources, volatile compounds also influence pollinator choices (Proctor et al. 1996[30]). Bees and butterflies, which were the most frequent pollinators of the species studied, are said to locate distant food sources using scent-orientation (Harborne 1988[17], Proctor et al. 1996[30]). However, the flowers observed here did not present either odor or volatile compounds. Although neutral red solution stained the corolla lobes positively, it probably showed other floral glandular structures not related to scent production or areas with thin cuticle (Dafni 1992[7]). Thus bees and butterflies seem to be visually attracted to the flowers of the species studied, a kind of short-distance orientation used by these insects (Proctor et al. 1996[30]).

Psychotria jasminoides flowers have the greatest corolla length and are pollinated specially by Lepidoptera species, which have a long slender proboscis clearly adapted for reaching nectar on tubular flowers (Proctor et al. 1996[30]). Besides also presenting tubular flowers, the other species studied are mainly pollinated by the bees *T. spinipes* and *P. helleri*, which may be a consequence of their smaller corolla. Moreover, the flowering peak of *P. jasminoides* occurs at the beginning of the wet season, when flower availability is low at the study site (pers. obs.), thus this species may consist an important resource for butterflies at this period of the year, for which nectar is an important source of energy (Proctor et al. 1996[30]).

Floral diameter also seems to play an important role in determining the main pollinator groups of the study plant species. The larger diameter of *P. nuda* flowers seems to be more adapted to hummingbird pollination, since the hummingbird bill is thicker than the bees' tongue. In addition, smaller bees, such as *C.* cf. *mulleri*, were more frequently observed visiting *P. birotula* flowers, which present the smaller corolla diameter.

Plant-pollinator relations observed here are not specialized, since more than one pollinator species may pollinate each studied plant. Some authors suggest that pollinator identity (Khon and Barrett 1992[19]) and specialization (Olesen 1979[26], Björkman 1995[3]) may not be so important as generally assumed for distylous species. Relatively long mouthparts, like those of most pollinators recorded here, may reach lower sexual organs and be sufficient to perform legitimate pollination, maintaining the levels of intermorph matings (Khon and Barrett 1992[19]). Almost all important pollinators were observed contacting thrum anthers and pin stigmas with the same region of their body, which may result in mixed pollen loads.

The epipetalous stamens and tubular corolla presented by the species studied are frequently recorded in distylous plants, and are also typical of Rubiaceae flowers in general (Robbrecht 1988[33], Lloyd and Webb 1992a[24], b[25], Björkman 1995[3]). Tubular flowers force visitors to introduce their mouthparts fairly precisely at the bottom of the tube, contacting sexual organs before and after they reach the nectar, secreted at the base of the corolla (Björkman 1995[3], Stone 1995[36], but see Waser et al. 1996[39]). These aspects maximize pollen transfer per visit, and may be an important factor influencing legitimate pollinations in distylous species, since the proportion of pollen delivery on compatible stigmas is frequently low in these plants (Stone 1995[36]).

Pin flowers were more frequently visited than thrum ones in almost all of the species studied. This result may indicate the occurrence of the asymmetrical pollen flow generally observed towards pin morph (Barrett 1992[1]), which may be a consequence of the exerted positioning of pin stigmas, making them more easily contacted by the pollinator's body (Dulberger 1992[9]). As observed in this study, the bees were more frequently observed collecting pollen of thrum anthers, a behavior that which may also contribute for this asymmetrical pollen flow.

Nevertheless, higher frequency of visits does not necessarily implies in higher deposition of pollen grains on the stigma, neither to legitimate pollination. In fact, besides receiving less pollen grains, thrum flowers generally receive a higher proportion of compatible ones in relation to pin flowers (Barrett 1992[1]), and thus are expected to set more fruits, as observed in *P. jasminoides* (Castro and Oliveira, unpublished data). The larger corolla of thrum flowers, as observed here, is generally interpreted as compensate this asymmetrical pollen flow, resulting in a greater attractiveness for pollinators in relation to the smaller corolla of pin flowers (Dulberger 1992[9]). The higher concentration of sugars in the nectar of these flowers may also maximize pollinator attractiveness.

Besides the interaction between corolla length, number of visits and fruit-set, as suggested above for *P. jasminoides*, our results show that this interaction is not the rule. In *P. mapourioides*, whose thrum corolla in larger than pin ones, frequency of visits between morphs is similar, and fruit-set in thrums is higher. On the other hand, pin flowers of *P. birotula* receive more visits and set more fruits than do thrum ones, in spite of presenting similar corolla length. Finally, pin flowers of *P. nuda* are more frequently visited and set similar number of fruits to thrum ones, in spite of having a smaller corolla.

Almost all pollinator species observed here have similar foraging strategies, *e.g.*, fly long distances between flowering patches (Proctor et al. 1996[30]), as the hummingbirds, which behaved as trapliners, visiting flowers in constant intervals and following a given rout (Feinsinger and Colwell 1978[10]). Pollinator foraging strategy plays a direct influence on pollen flow and fruit set of distylous species, since intermorph pollen flow is necessary for reproductive success of these plants (Dulberger 1992[9]).

Besides being capable of fly long distances and therefore perform intermorph pollination, the main pollinators of the studied plant species were observed visiting several flowers of each inflorescence, thus maximizing self-pollination. In this situation, self-incompatibility system of the species studied (Castro and Oliveira, unpub. data) is an important strategy to avoid autogamy through geitonogamy.

The sequential flowering of the study species may contribute to the maintenance of *T. spinipes* and *P. helleri* in the study area, since they were observed visiting all plant species. Different flowering periods along the year may also avoid interespecific pollination by these bees. The overlap between *P. birotula* and *P. mapourioides* flowering periods may be minimized by the different habitat occupied by these species, e.g., the slope and coastal plain, respectively. Nevertheless, data on flowering phenology may be interpreted with care, because it is generally variable from one year to another, since it is highly influenced by abiotic conditions (Gajardo 1997[12]). Some pollinators as *Parides tros* are considered rare, and this was the first report of this butterfly for São Paulo state (K. S. Brown, pers. comm.).

Pollination patterns recorded here are similar to those generally observed in tropical communities (Kress and Beach 1994[19]). Highly specialized flowers are rare in Rubiaceae, and bees are the commonest floral visitors, together with the presence of other groups of pollinators, such as butterflies and hummingbirds. As shown here, bees are legitimate visitors except when floral morphology prevents them from reaching the nectar; in these situations, some species are simply excluded from nectar feeding, whereas others may rob nectar or collect pollen directly from the anthers (Proctor et al. 1996[30]). In this study, the restriction presented by the plants to the bees seems to be the corolla length and diameter, distribution of individuals and flower availability throughout the year.

Despite some restrictions on pollen flow, related to pollinator behaviour, the species studied seem to set seed to maintain their populations in the study area. This result may be achieved through the interaction between pollination biology and the self- and intramorph-incompatibility observed in these plants (Castro and Oliveira, unpublished data).

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CAPÍTULO II

Sistema reprodutivo e morfometria floral de Rubiáceas distílicas de Mata Atlântica

Breeding system and floral morphometry of distylous Rubiaceae from the Atlantic rain forest, SE Brazil[◆]

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Running title, Distyly in Rubiaceae from Atlantic rain forest

Key words: Atlantic forest, SE Brazil, distyly, floral morphometry, floral dimorphism, herkogamy, mating system, morph ratio, pollination, *Psychotria*, reproduction, Rubiaceae, self-incompatibility.

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Abstract. General patterns of floral morphology and incompatibility mechanisms have been described for many distylous plants. Their absence in typically distylous groups, as observed specially in tropical environments, is interpreted as atypical distyly, or as a new reproductive strategy derived from it. Data presented here constitute a study on the morphological and compatibility relations between floral morphs of Rubiaceae dimorphic species, from the Atlantic rain forest at SE Brazil: *Psychotria jasminoides*, *P. birotula*, *P. mapourioides*, *P. pubigera* and *P. nuda*. Hand pollination experiments, floral organ measurements and morph ratio estimates were performed. Three species are typically distylous, with reciprocal herkogamy, self- and intramorph-incompatibility and a balanced morph ratio in the population; two species are atypical, because they lack one of these features. Floral organs are larger in thrum flowers, and incompatible pollen tubes stop growing in the stigma or upper parts of the style. Results indicate that most species studied present functional distylous syndrome expected in stable populations.

INTRODUCTION

Distyly is a balanced floral dimorphism genetically related to a sporophitic incompatibility system, first recognized by Darwin (1877 *apud* Ganders 1979), in which two floral morphs have complementary positioning of anthers and stigma. Thrum flowers have longer anthers and a short stigma, and pins have the opposite (Barrett 1992). This reproductive strategy is considered a case of morphological and physiological convergence, acting as an anti-selfing device that maximizes both male and female fitness (Ganders 1979, Charlesworth and Charlesworth 1979, Webb and Lloyd 1986, Lloyd and Webb 1992 a, b, Dulberger 1992, Stone 1995). Several families may present distylous species (Ganders 1979, Murray 1990, Barrett 1992 and references therein), however the great majority of distylous genera are included in the family Rubiaceae (Robbrecht 1988, Barrett 1992), especially in the tribe Psychotrieae, in which distyly is considered a primitive characteristic (Hamilton 1990).

Reciprocal herkogamy, self- and intramorph-incompatibility and equilibrated morph ratio (isoplethy) are features generally observed in distylous species (Ganders 1979, Barrett 1992). Floral asymmetry between morphs such as larger corolla, anther and stigma lobes of thrum flowers, greater pollen production in pins, and polymorphisms in pollen size, color and exine sculpture, and stigma are secondary traits generally, but are not always, recorded (Sobrevilla et al. 1983, Barrett and Richards 1990, Richards and Koptur 1993, Pailler and Thompson 1997, Herman et al. 1999).

Several studies indicate a number of possible deviations from these morphological and mating patterns in typical distylous taxa, such as absence of reciprocal herkogamy (Hamilton 1990, Dulberger 1992), presence of monomorphy (Bawa and Beach 1983, Barrett and Shore 1987), homostyly (Barrett and Shore 1987, Washitani et al. 1994), different degrees of incompatibility strength (Philip and Shou 1981, Bawa and Beach 1983), self-compatibility (Richards and Koptur 1993) and dioecy (Ross 1982, Pailler et al. 1998). Plants presenting these traits may not be considered typically distylous, but species in which a new reproductive strategy arose from distyly (Hamilton 1990, Pailler and Thompson 1997).

Tropical species are more likely to present such deviations, probably due to the great diversity of selective forces acting upon distylous syndrome, like pollinator species (Barrett and

Richards 1990), flowering phenology and habitat (Björkman 1995, Pailler et al. 1998). Many studies concerning distyly include tropical Rubiaceae, a family that constitutes one of the most important understory plant groups of Neotropical forests (Gentry and Emmons 1987, Laska 1997). Examples of such studies are Sobrevilla et al. (1983), Perez-Nasser et al. (1993), Richards and Koptur (1993), Stone (1995, 1996), Pailler and Thompson (1997), Ree (1997) and Pailler et al. (1998). However, little information on distylous Rubiaceae species from the Atlantic rain forest is available (*e.g.*, Passos and Sazima 1995).

The objectives of this study are a) to investigate floral morphology and compatibility relations of understory Rubiaceae shrubs presenting floral dimorphism in an area of the Atlantic rain forest of southeastern Brazil; b) to relate these results with morph ratio and natural fruit-set within studied populations; c) to determine if the species studied should be considered as typically distylous.

MATERIAL AND METHODS

Study area and species studied

The study was conducted from August 1997 to May 2000, in the Parque Estadual da Serra do Mar, Núcleo de Desenvolvimento de Picinguaba, Ubatuba, São Paulo State, SE Brazil (44°48' - 44°52W and 23°20'- 23°22'S, Fig. 1A, p8). Atlantic rain forest is part of the Atlantic forest and occurs along SE Brazilian coast, being one of the most diverse environment of the world, including coastal plain and slope areas, which constitute distinct environments also encountered at the study site. Nowadays, Atlantic rain forest distribution has been highly restricted by human activities (Viana and Tabanez 1997). The climate of Serra do Mar, where the study site is located, is tropical wet (*sensu* Walsh 1996) or tropical rainy (*sensu* Köppen 1948) with rain occurring every month (Fig. 1B, p.8).

The five species studied were the shrubs *Psychotria mapourioides* DC., located in the coastal plain area, *Psychotria jasminoides* (Cham.) Müll. Arg., *P. birotula* L. B. Sm. & Downs and *P. pubigera* Schltdl., which are mainly found in the slope area (Fig. 1C, p.8). Data on *P. nuda* (Cham. & Schelet.) Wawra, encountered at both sites (Fig. 1C, p.8), were collected by Castro and Araujo (unpublished data) in the coastal plain in 1994 and will be used here for comparison.

Flowering peaks of the study species are sequential throughout the wet season, *P. jasminoides* flowers in October, *P. mapourioides* and *P. birotula* in November, *P. pubigera* in December and January and *P. nuda* from March to April (Castro and Oliveira 2001, unpublished data). As in most species of Rubiaceae, flowers have synsepalous calyx, synpetalous, tubular corolla, an isostemonous androecium with partially adnate stamen filaments, inferior and syncarpic ovary, and a basal nectariferous disc (Castro and Oliveira 2001, unpublished data). Flowers are pale-colored (except *P. nuda*), diurnal and last one day. *Psychotria jasminoides* is mainly pollinated by butterflies, *P. birotula*, *P. mapourioides* and *P. pubigera* by bees, and *P. nuda* by hummingbirds (Castro and Oliveira, unpublished data).

Floral morphometrics

Floral morphometric data were collected using a digital caliper (error, 0.01 mm) on a sample of 30 flowers per morph per species (preserved in FAA 50), randomly collected in a minimum of ten individuals of each morph, from up to two populations each species, following Richards and Koptur (1993). All measurements were compared between the two morphs with a student t test (Sokal and Rohlf 1981).

Exine sculpture of *P. jasminoides* thrum and pin pollen was studied using scanning electron microscopy. Material was preserved in a 3% phosphate buffer, washed with H_2O_2, dehydrated in a graded cetonic series, critical point dried in a Ladd CO_2 critical-point dryer, and viewed at a JEOL/JSM - T3000 Scanning Microscope.

Reproductive experiments

Compatibility relations within and between floral morphs were evaluated through fruit set comparison and pollen tubes observation after hand-pollination experiments. Five types of reproductive experiments were performed for each morph, self-, intramorph- (illegitimate) and intermorph (legitimate)-pollinations, apomixis and control. We used twenty marked individuals of each morph, in a minimum of two populations of each species; for each experiment a minimum of 28 flowers was used from three to five individuals. The number of hand-pollinated flowers per inflorescence did not exceed mean fruit-set/inflorescence, and most untreated flowers in treated inflorescences were left unpollinated.

For reproductive experiments, inflorescences containing unopened flowers were enclosed in mesh bags; we did not emasculate the buds, which are very small, in order to avoid stigma damage (except in apomixis experiments). Although many authors suggest that the interaction between pollen tubes from legitimate and illegitimate crosses would be important, experimental studies show that such interaction seems to have little effect on pollen tube performance (Murray 1990, Khon and Barrett 1992, McKenna 1992, Stone 1995, Nishihiro and Washitani 1998). Some studies showed that pollen tubes of legitimate crosses may easily out compete the illegitimate ones in heterostylous plants (Murray 1990, Khon and Barrett 1992, McKenna 1992, Stone 1995, Nishihiro and Washitani 1998). Recently opened flowers were emasculated immediately before hand pollination experiments, and pollinated by brushing a dehiscent anther across the stigma. Most of the pollen used in intra- and intermorph-pollinations was collected from flowers bagged only for this purpose, minimizing the possibility of contamination.

Flowers subject to different treatments were color coded with a drop of acrylic paint near the pedicel and covered until flower senescence, when bags were removed. Fruits that resulted from field experiments were accompanied monthly until they reached more than a half of the normal size of a mature fruit, which occurred more or less two months after pollination, and one month before total maturation. Fruit set comparisons between experiments and morphs from the same species were performed using Chi-square tests. Pollen tube growth of all the experiments described above (except apomixis and control) was observed using the fluorescence technique (Martin 1959) in pistils fixed 12, 24, 48 and 72 h after hand pollination. It was used a minimum of ten pistils/treatment/time interval.

For morph ratio estimates, all individuals of each morph were counted in an area of ca. 2000 m^2. Voucher specimens of studied plants were deposited at the Universidade Estadual de Campinas herbarium (UEC 118309-118315).

RESULTS

Flowers of all species studied are reciprocally herkogamous, except those of *P. nuda*, in which herkogamy is not exactly reciprocal, e.g. anthers height is similar to the stigma height of the opposite (Table 1, Fig. 2, Fig. 3A, B). In Fig. 2 it is possible to note that stigma heights are almost completely separated between floral morphs (X axis), thus statistically different (Table 1), whereas anther heights are overlap somewhat (Y axis), but are still statistically different (Table 1). *Psychotria nuda* presents the greatest overlapping in anther height between floral morphs, which are similar (Fig. 2, Table 1).

Thrum flower organs are generally larger than pin ones, having longer anther and corolla lengths in *P. mapourioides* and *P. nuda*, stigma lobe length in *P. jasminoides*, *P. pubigera* and *P. nuda* and corolla diameter in *P. nuda*. The last species presents the greatest differences between morphs. Moreover, stigma lobe and anther lengths are the most variable traits, whereas corolla diameter is the most conservative one (Table 1).

The exine sculpture is different between thrum and pin morphs of *P. jasminoides*. Although both pollen sculptures are reticulate, in the thrum form it is more delicate, with globular expansions that are absent in pin flowers (Fig. 3).

Almost all species present higher fruit-set after cross-pollination greater than after self-, intramorph-pollination and apomixis treatments ($P<0.05$ for all), indicating that they are self- and intramorph-incompatible. In *P. birotula*, thrum fruit-set after intermorph-pollination was similar to self-pollination and lower than apomixis experiments ($P<0.005$ for both, Table 2), showing that the species may set fruit through apomixis. Fruit production of control flowers, compared with legitimately pollinated ones, varies between species (Table 2), most are lower (*P. jasminoides* thrums and pins, *P. birotula*, pins, *P. mapourioides*, pins, $P<0.005$ for all), and just a few are higher (*P. mapourioides*, thrums, $P<0.025$ and *P. birotula*, pins, $P<0.005$) or similar (*P. nuda*, thrums and pins, $P<0.005$). These results shows that, although general patterns of incompatibility mechanisms are observed, each species may present particular features that will be discussed above.

Incompatibility reaction was clearly observed under fluorescence microscopy. The great majority of legitimate pollen tubes arrive at the base of the style 12 h after pollination (Fig. 3E); in some cases it was possible to observe them inside the ovary and near the ovules (Fig. 3F). Illegitimate pollen tubes germinated massively on the stigma in both floral morphs, and generally presented callose plugs of irregular size and slightly swollen tips which fluoresce brightly after staining with aniline blue (Fig. 3G, H). A low proportion of them may grow into style tissue, mainly in pin flowers.

TABLE 1. *Morphometrics of floral traits of Psychotria species in the Atlantic rain forest, SE Brazil (mm, Mean ± SD values).* Pj: *P. jasminoides*, Pb: *P. birotula*, Pm: *P. mapourioides*, Pp: *P. pubigera*, Pn: *P. nuda*, T: *thrum flowers*, P: *pin flowers*, *t / P:* t *and* P *values of student tests, respectively* (*P<0.01; **P<0.05; ns: non-significant differences).

Sp	Floral	Stigma		Anther[b]		Corolla	
	morph	Height	Lobe length[a]	Height	Length	Length	Diameter
Pj	T	7.6 ± 0.84	2.1 ± 0.4	11.3 ± 1.32	2.2 ± 0.4	8.8 ± 0.97	1.8 ± 0.33
	P	11.6 ± 1.18	1.7 ± 0.4	7.6 ± 0.66	2.0 ± 0.3	8.5 ± 0.73	1.7 ± 0.24
	t / P	-14.83 *	3.58 **	13.74 *	1.45 *ns*	1.39 *ns*	1.52 *ns*
Pb	T	2.7 ± 0.23	0.8 ± 0.24	4.4 ± 0.33	1.3 ± 0.1	3.2 ± 0.26	1.5 ± 0.2
	P	4.6 ± 0.32	0.7 ± 0.12	2.7 ± 0.21	1.1 ± 0.09	3.1 ± 0.33	1.3 ± 0.2
	t / P	-26.1 *	2.2 *ns*	23.23 *	7.82 *	1.12 *ns*	2.48 *ns*
Pm	T	6.3 ± 0.6	1.5 ± 0.3	9.0 ± 0.6	1.8 ± 0.23	7.0 ± 0.45	2.0 ± 0.25
	P	8.7 ± 0.49	1.3 ± 0.18	6.0 ± 0.3	1.5 ± 0.11	6.4 ± 0.58	1.9 ± 0.13
	T /P	-11.86 *	2.27 *ns*	17.24 *	5.33 *	3.24 **	1.15 *ns*
Pp	T	6.0 ± 0.61	1.7 ± 0.4	8.5 ± 0.61	2.2 ± 0.26	7.2 ± 0.71	1.6 ± 0.24
	P	9.2 ± 0.58	1.1 ± 0.23	7.1 ± 0.4	2.3 ± 0.12	6.9 ± 0.41	1.6 ± 0.19
	t / P	-20.75 *	7.21 *	10.62 *	-1.96 *ns*	1.77 *ns*	52.5 *ns*
Pn	T	10 ± 0.92	2.7 ± 0.45	17.8 ± 1.76	23.5 ± 1.94	5.5 ± 0.49	6.8 ± 0.53
	P	19.2 ± 1.52	1.8 ± 0.6	13.7 ± 1.22	21.4 ± 1.83	4.7 ± 0.44	5.8 ± 0.7
	t / P	-28.4 *	6.54 *	10.54 *	6.68 *	4.28 *	6.19 *

[a] 1 stigma lobe/flower

[b] 1 anther/flower

Morph ratio (thrum:pin) for each species were 45:34 (1.4:1) for *P. jasminoides e P. mapourioides*, 11:13 (1.1:1) for *P. birotula* and 6:3 (2:1) for *P. pubigera*. All populations may be considered isopletic, except for *P. pubigera*, which is anisopletic.

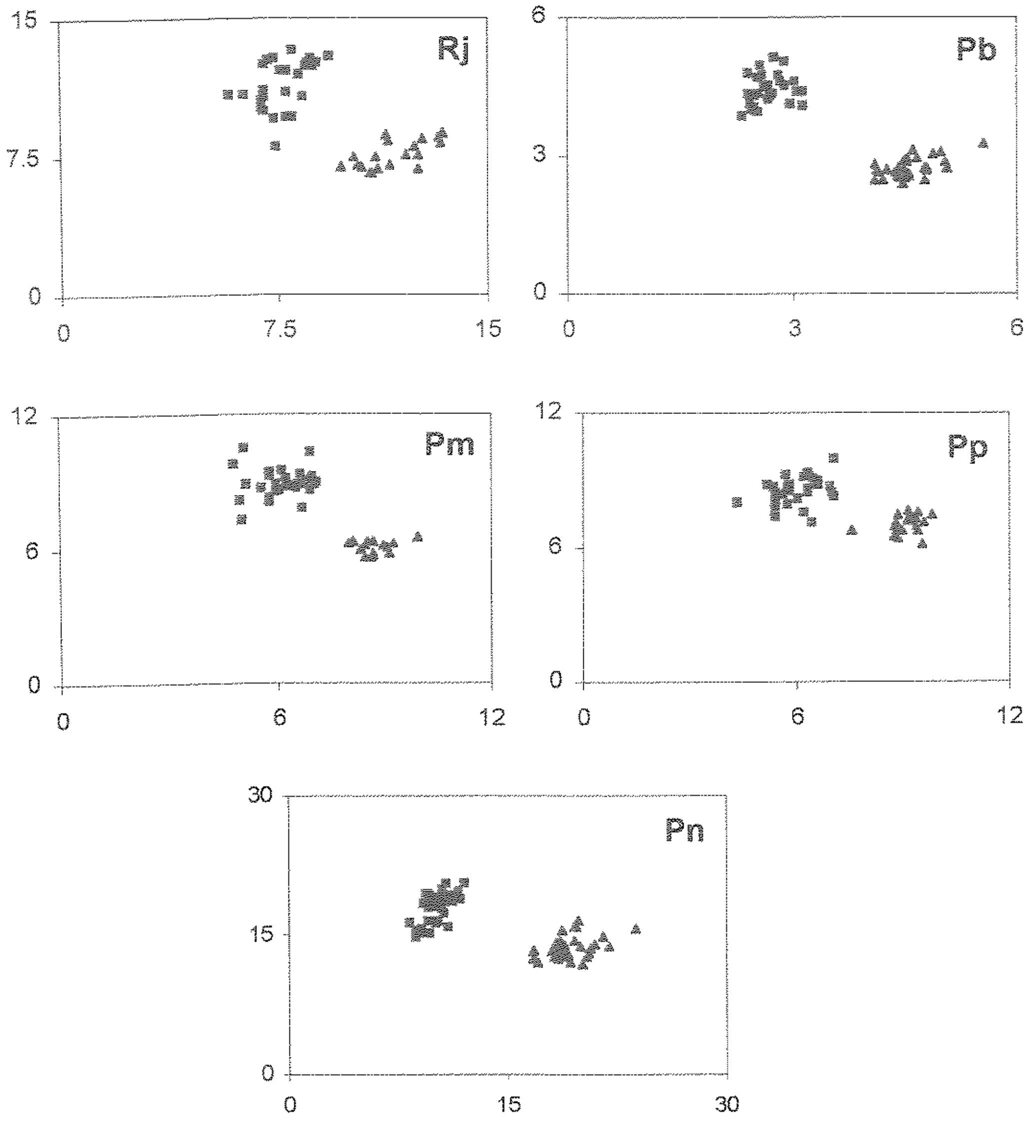

FIG. 2. Plot of stigma (x-axis) and anther (y-axis) heights (mm) of thrum (squares) and pin (triangles) flowers of *Psychotria* species in the Atlantic rain forest, SE Brazil. Pj, *P. jasminoides*, Pb, *P. birotula*, Pm, *P. mapourioides*, Pp, *P. pubigera*, Pn, *P. nuda*.

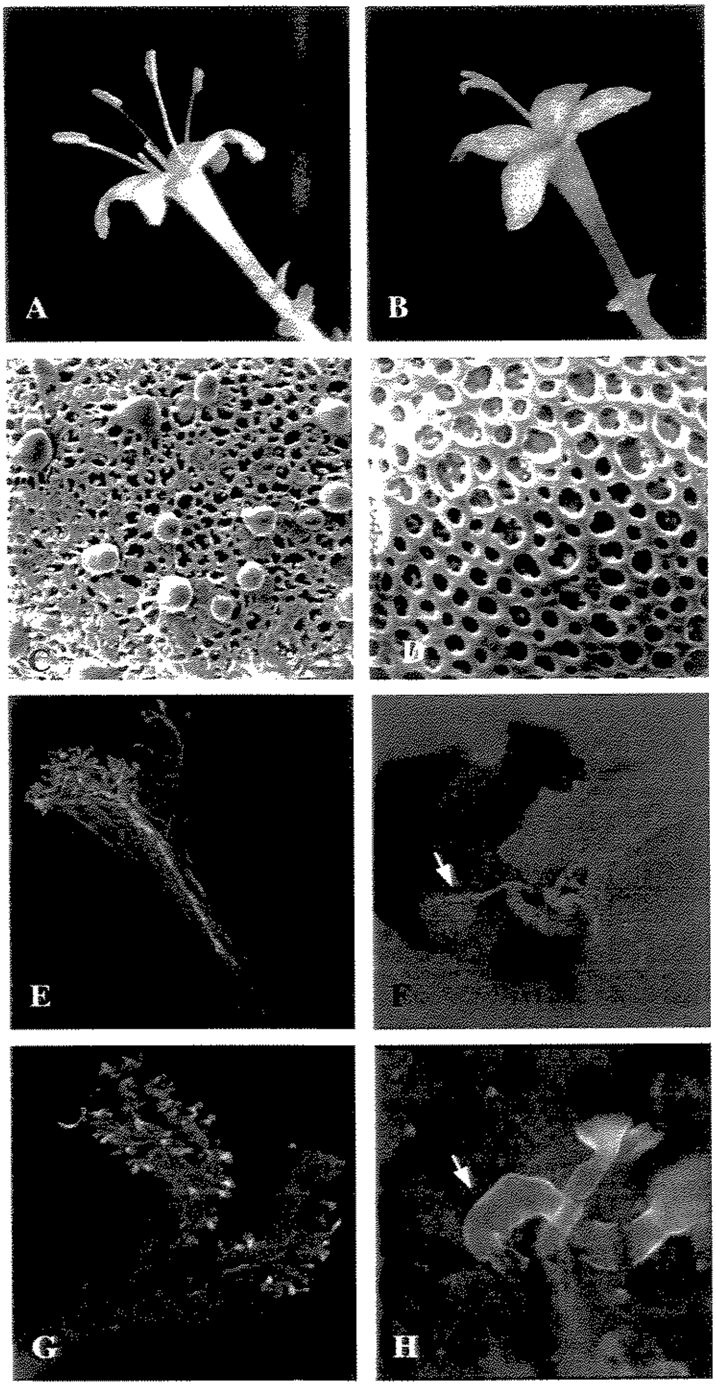

Figure 3. Flowers, exine sculpture and pollen tubes of *Psychotria* species in the Altlantic rain forest, SE Brazil. A, B. Thrum and pin morphs of *Rudgea jasminoides*, respectively. (Bar: 3 mm). C, D. Exine sculpture of *R. jasminoides* thrum (5000X) and pin (6750X) pollen grains, respectively. E, F. Compatible pollen tubes reaching the base of the style (4X) and near an ovule (arrow) of a pin flower of *Psychotria birotula* after intermorph-pollination (40X), respectively. G. Stigmatic incompatibility on thrum morph of *P. pubigera* after intramorph-pollination (4X). H. Callose deposition (arrow) that resulted from stigmatic incompatibility on thrum morph of *P. birotula* after self-pollination (10X).

TABLE 2. *Results of hand pollination experiments (% of fruits, number of flowers) on Psychotria species in the Atlantic rain forest, SE Brazil. Pj: P. jasminoides, Pb: P. birotula, Pm: P. mapourioides, Pn: P. nuda; S: self-pollination, CR: intermorph (cross)-pollination, IM: intramorph-pollination, AG: apomixis, CO: control; T and P: thrum and pin flowers, respectively.*

Sp	Morph	CR	S	IM	AG	CO
Pj	T	70.0 (40)	14.7 (34)	5.1 (39)	0.0 (50)	69.6 (46)
	P	72.1 (43)	7.5 (40)	6.0 (50)	0.0 (37)	25.6 (39)
Pb	T	5.9 (34)	9.5 (42)	0.0 (40)	9.3 (43)	5.7 (70)
	P	13.5 (37)	0.0 (43)	0.0 (36)	3.9 (51)	25.6 (82)
Pm	T	17.9 (28)	0.0 (35)	0.0 (32)	0.0 (35)	40.0 (30)
	P	35.5 (31)	3.4 (29)	3.4 (29)	2.4 (41)	13.3 (30)
Pn	T	54.0 (37)	3.1 (32)	13.5 (37)	6.8 (44)	55.2 (29)
	P	42.9 (28)	0.0 (30)	0.0 (22)	7.7 (39)	53.0 (49)

DISCUSSION

The species studied may be divided into two categories, typically distylous (*P. jasminoides*, *P. birotula* and *P. mapourioides*) and atypically distylous (*P. pubigera* and *P. nuda*). Typically distylous species considered here are those with reciprocal herkogamy, self- and intramorph-incompatibility and isopletic populations (*e. g* morph ratio near to 1,1); atypical ones are those that lack one of these features (Hamilton 1990, Barrett 1992). *Psychotria pubigera* does not present an isopletic population, and *P. nuda* lacks reciprocal herkogamy. Although these species do not have one of those typical features cited above, it is possible that their reproductive strategies are derived from distyly, since this mechanism is the most common within their tribes (Robbrecht 1988, Hamilton 1990). Some authors suggest thet the species lacking an exactly reciprocal hercogamy may also be considered distylous ones (Faivre and McDade 2001)

Data on *P. jasminoides*, *P. birotula* and *P. mapourioides* may indicate that reciprocal herkogamy promotes intermorph pollination in distylous plants (Barrett and Richards 1990, Khon and Barrett 1992, Lloyd and Webb 1992b, Stone 1995). These species are reciprocally herkogamous, legitimately fertilized in nature (*e.g.*, the majority of control flowers set fruits in a similar way to those legitimately pollinated), present the classical distylous breeding system and have isopletic populations. On the other hand, although anther height of different morphs of *P. nuda* overlap somewhat, control flowers had a relatively high fruit set, showing that in this case the absence of an exactly reciprocal herkogamy may not prevent legitimate fertilization.

However, these interpretations must be true only if one consider that the breeding system is working as expected for distylous species. Our results of the reproductive experiments show that all species present some fruit production after self-pollination and, except in the case of *P. jasminoides*, after apomixis. Thus fruit-set after naturally pollinated flowers may include intermorph-, self-pollination and asexual reproduction or apomixis. Although apomixes has not been recorded for Rubiaceae species, it may occurs among tropical plants in general, being advantageous because when fix an extremely fit genotype, and when assure reproduction in the absence of pollination (Richards 1997).

The population of *P. pubigera* was not isopletic, despite the presence of morphological and mating system features similar to those of the typical distylous species studied. Anisoplethy in distylous plants is generally interpreted as a consequence of a breakdown of the incompatibility system of the more abundant floral morph (Ganders 1979, Barrett 1992). Nevertheless, pollen tube data of *P. pubigera* clearly show strong self- and intra-morph incompatibility. Population distribution of this species included a clearing area where it was very difficult to locate individuals because a large tree fell and covered many plants; thus, we suggest that pin individuals may have been underestimated if this morph presents a clumped distribution.

This study, like others including Rubiaceae genera such as *Rudgea*, a genus closely related to *Psychotria* (Baker 1956 *apud* Hamilton 1990), *Palicourea* (Sobrevilla et al. 1983), *Luculia* (Murray 1990) and *Cephalanthus* (Richards and Koptur 1993), showed thrum flowers with larger floral organs (excluding style length). This feature has been observed in many other groups (Ganders 1979, Dulberger 1992). However, corolla size is similar between floral morphs of the species studied here. Several of these distylous floral asymmetries have been interpreted as a compensation for the asymmetric pollen flow generally observed on pin stigmas; thus the larger corolla of thrum flowers might attract more pollinators than that of pin flowers (Ganders 1979, Barrett and Glover 1985, Dulberger 1992, Björkman 1995). However, some authors suggest that this feature may be also related to the longer stamen filaments of thrum flowers, since stamens development is phylogenetic and ontogenetically related to that of the corolla (Ganders 1979, but see Richards and Koptur 1993).

The majority of species studied here have larger thrum stigmas, similarly to those observed in *Palicourea* (Sobrevilla et al. 1983). Differences in stigmatic lobes are considered rare and, when recorded, pin stigmas are generally larger (Dulberger 1992). Nevertheless, differences in stigma size were recorded in *Guettarda* (Richards and Koptur 1993), *Hedyotis* (Ornduff 1980), *Rudgea* (Baker 1956 *apud* Dulberger 1992) and *Psychotria* (Baker 1958 *apud* Murray 1990). As observed here, several floral features relationships between morphs may be observed, probably because of the polyphyletic origin of distyly in Rubiaceae (Murray 1990, Dulberger 1992). The adaptive significance of these secondary floral features is not clear. Discussion of theoretical and experimental aspects on this matter may be found in Murray (1990), Dulberger (1992), Khon and Barret (1992), Lloyd and Webb (1992a, b).

Differences in pollen exine sculpture, as those observed here, is generally recorded in Rubiaceae species (Bir Bahadur et al. 1984a *apud* Dulberger 1992), and are interpreted as a developmental outcome of size dimorphism generally observed between thrum and pin pollen grains (Dulberger 1992 and references therein).

Mating systems of the species studied are similar to what would be expected for distylous plants. Thrum flowers seems to have stronger incompatibility systems, because incompatibility reaction occurs more frequently at the stigma, while in pin flowers it may occur in the stylar tissue. This result is generally interpreted as a strategy to avoid illegitimate fertilization, since in thrum flowers there is a shorter distance along which pollen tubes must grow to reach the ovary (Dulberger 1992). Bawa & Beach (1983) also recorded different sites of inhibition of illegitimate pollen tubes in several species of tropical Rubiaceae.

Slight variation in the breeding system between species in a genus, as observed here for *P. pubigera*, is commonly observed in plants (Sobrevilla et al. 1983), and may also occur between morphs of distylous species (Washitani et al. 1994). *Psychotria*, for example, is a genus with a wide spectrum of breeding systems (Sobrevilla et al. 1983, Hamilton 1990), and it is not surprising that some deviations are observed, especially in tropical environments, where pressures upon reproductive traits may be more varied (Barrett and Richards 1990, Björkman 1995, Pailler et al. 1998).

The variable fruit-set of control flowers within and between species, as observed here for *P. birotula* and *P. mapourioides*, suggests that, although the majority of species were legitimately pollinated, in some of them this was not the case. Washitani et al. (1994) also recorded a variable pattern of fruit-set within and between individuals of a distylous *Primula* species. Low efficiency of legitimate pollinations (indicated by low fruit set under natural conditions) is not uncommon (Björkman 1995), but low fruit/flower ratio, as recorded here for *P. birotula, P. mapourioides* and *P. pubigera* is generally interpreted as a consequence of the high flower production through which plants maximize male function (Stone 1995).

We conclude that, at the study site, the distylous syndrome presents some variation in typical patterns, as the breeding system and morph ratio presented by *P. pubigera* and non-exactly reciprocal herkogamy in *P. nuda*. Nevertheless, the majority and most important morphological and mating features of distyly seems to be preserved, since most of the species studied present the distylous syndrome working as expected in stable populations.

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CAPÍTULO III

Hercogamia em uma Rubiácea de Mata Atlântica

Herkogamy in *Chiococca alba* (Rubiaceae) in the Atlantic rain forest, SE Brazil

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INTRODUCTION

Despite the relatively constancy of reproductive strategies in some tribes of Rubiaceae, such as distyly in Psychotrieae (Barrett 1992) and stylar pollen presentation in Gardenieae (Robbrecht 1988), other tribes may include both strategies, as Chiococceae (Robbrecht 1988), or reproductive traits derived from them, as Cinchoneae (Castro and Oliveira 2001).

Herkogamy is the spatial separation between male and female sexual organs of the flowers. There are several types of herkogamy, including homomorphic (ordered and unordered) types and heteromorphic ones. These reproductive strategy is interpreted as a mechanism that promotes outcrossing besides avoiding self-pollination and mutual interference between sexual functions, *e.g.*, pollen dispatch and receipt (Webb and Lloyd 1986 and references therein).

The objective of this study is to investigate the reproductive biology of *Chiococca alba* (L.) Hitchc. (Chiococceae), a vine widely distributed along North, Central and South America (Andersson 1992).

MATERIAL AND METHODS

The study was conducted between February 1999 and February 2000 in the Parque Estadual da Serra do Mar, Núcleo Picinguaba, on the southeastern Brazilian coast (44°48' - 44°52W and 23°20' - 23°22'S, Fig. 1A, p.8), in an area of coastal plain of the Atlantic rain forest. The climate of Serra do Mar, where the study site is located, is tropical wet (*sensu* Walsh 1996[1]) or tropical rainy (*sensu* Köppen 1948) with rain occurring every month (Fig. 1B, p.8).

Phenology data were collected for 20 individuals during one year, following Fournier (1974). Time of anthesis, floral longevity and nectar secretion were checked through daily monitoring of ten tagged flowers from seven individuals. Stigma receptivity was recorded by the peroxidase technique (Kearns and Inouye 1993) and the presence of osmophores with a neutral red solution (Dafni 1992). Nectar secretion was evaluated with glass capillaries, for a minimum of five flowers from a minimum of three individuals for each time interval (early and late morning, midday, afternoon and night), until 20:00 h.

Breeding system was evaluated through fruit set comparisons (using chi-square tests, Sokal and Rohlf 1981) after hand-pollination experiments (self-, cross-, control-pollination and apomixis). Observation of pollen tube growth in pistils fixed after hand-pollination experiments was done using fluorescence microscopy (modified from Martin 1959).

Floral visitors were survey by through focal observations from 6:00 to 22:00 h, totalling 16 hours of observation divided in different days, recording their behavior on the flower, including contact with stigma and anthers, kind of reward collected, interactions with other visitors, and the number of visits. It was not possible to collect any visitor, because of their very low frequency of visits and rapid movements. Thus, their morphological and behavioral features were recorded, and identification was made as far as possible.

Voucher specimens of the species was deposited at the Universidade Estadual de Campinas herbarium (UEC 118309-118315).

RESULTS

A flowering peak occurs in February (middle of the wet season, Fig. 2), and is highly synchronous between and within individuals. The axillary verticillate inflorescences (Fig. 3A) bear pentamerous, tubular, pendant flowers with a yellowish corolla and a green synsepalous calyx (Fig. 3A, B). Osmophores are present especially on the corolla lobes. The gynoecium includes a bilobate, exerted stigma (Fig. 3A) with a large receptive area (Fig. 3C), narrow style and an inferior, biloculate ovary, which bears two ovules. The stamens are inserted in the corolla tube (Fig. 3B), and produce large amounts of pollen.

Flowers open in the afternoon and wilt at the same time during the third day. The large receptive area of the stigma is active during the floral lifespan, and the powdery pollen is released at the pre-anthesis stage; by the morning of the day following anthesis, pollen had usually been removed. No nectar production was recorded, however, it was observed a nectary-like structure above the ovary, similarly to the majority of Rubiaceae species.

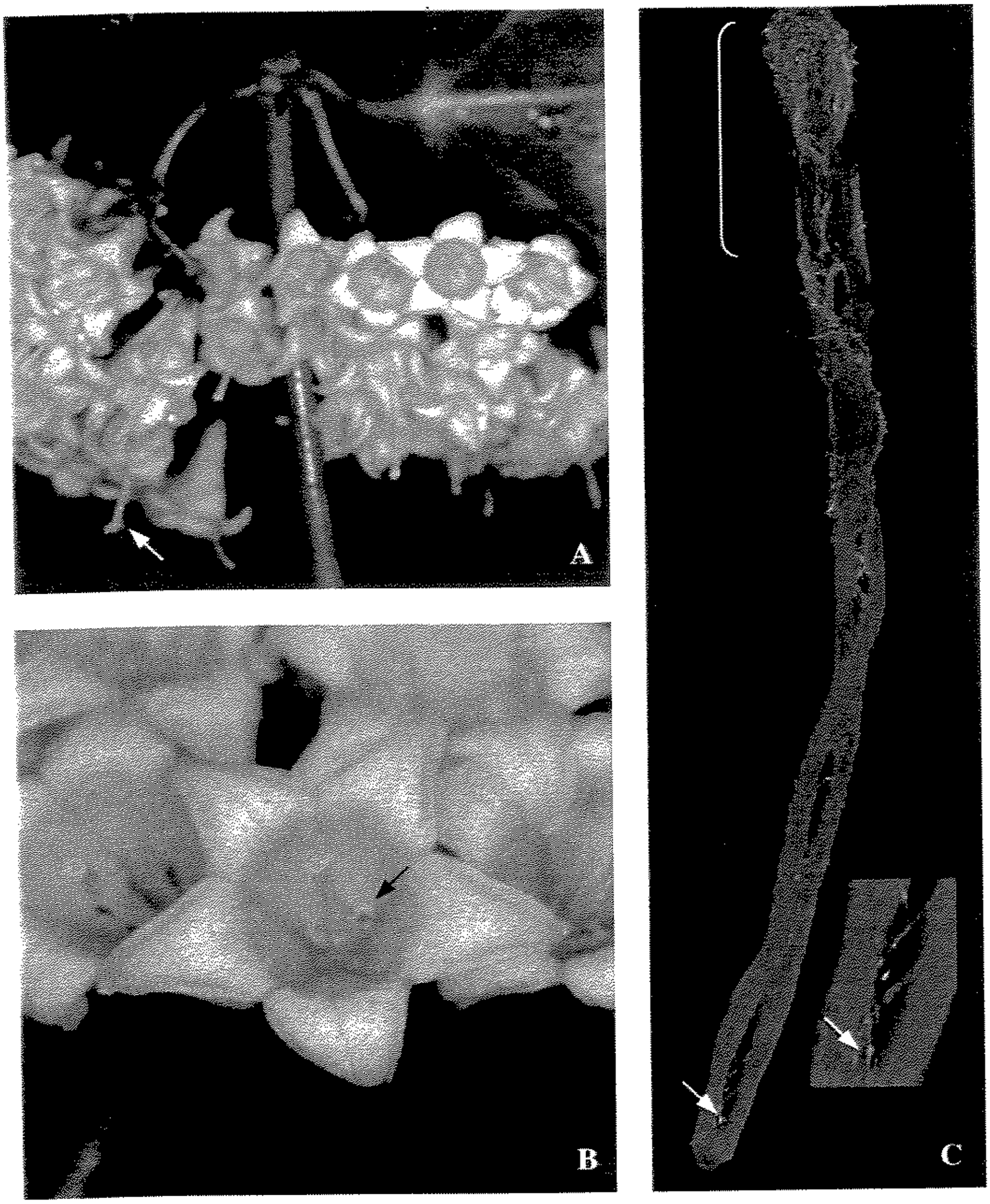

Figure 3. Reproductive features of *Chiococca alba* in the Atlantic rain forest, SE Brazil. A. Axillary verticillate inflorescences; note the exerted stigma (arrow). B. Flowers; note the inserted stamens (arrow). Bar: 0.15 mm. C. Compatible pollen tubes (arrows) reaching the base of the style (4X) 12 h after cross-pollination; note the large stigma (white line).

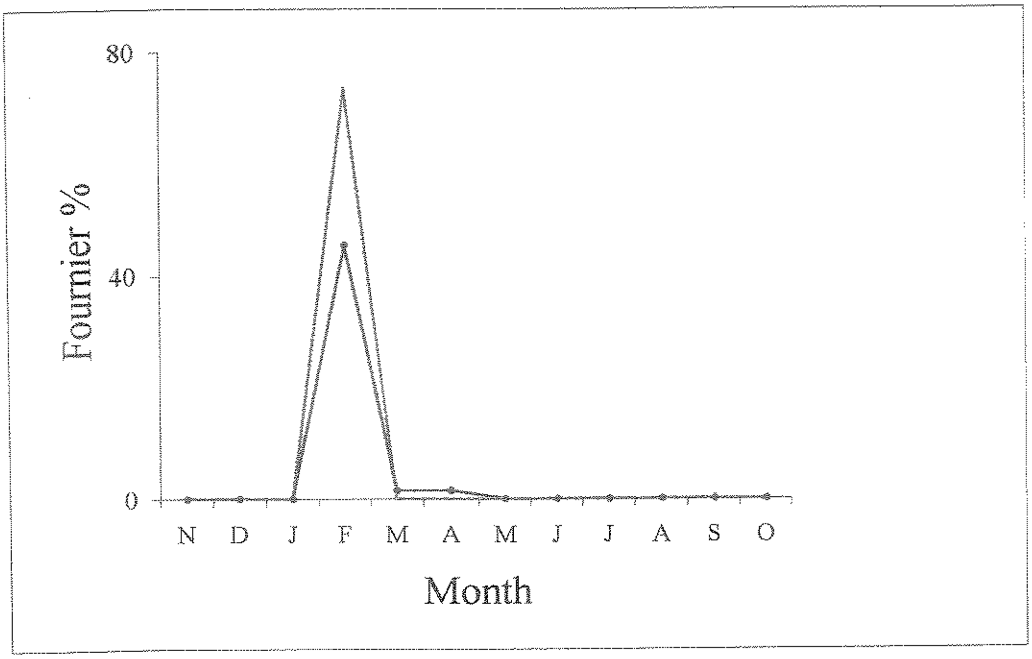

Figure 2. Fournier percentage of buds (red) and flowers (blue) of *Chiococca alba* at the Atlantic rain forest, SE Brazil.

Table 1. Results of reproductive experiments on *Chiococca alba* in the Atlantic rain forest, SE Brazil.

Experiment	% of Fruits (Flowers)
Self-pollination	12.5 (40)
Cross-pollination	61.54 (39)
Apomixis	5.55 (36)
Control	53.5 (43)

Visits to *C. alba* flowers were mainly by Lepidoptera and Hymenoptera species (Table 1), mainly between 14,00 to 16,00 h. Because of their very fast visits, it was not possible to observe their body contact with the flowers' sexual structures, except in the case of a species of medium bee a Lepidoptera, which were observed contacting the stigma when they seemed to collect pollen, since no nectar production was observed. Species of Diptera visited sporadically, when made legitimate visits or introduced the proboscis into holes at the base of *C. alba* floral tube, probably inflicted by other visitors.

Table 2. Visitor, orders and total number of visits to flowers of *Chiococca alba* at the Atlantic rain forest, SE Brazil.

Order	Number of visits
Lepidoptera	23
Hymenoptera	20
Diptera*	11

Chiococca alba is a self-incompatible ($\chi^2 = 6.40$, $P<0.025$, Table 2), non-apomitic species, with fruit-set after natural pollination similar to that after cross-pollination ($\chi^2 = 1.28$, $P<0.005$, Table 1). Incompatible pollen tubes stop growing in the stile, and compatible ones take 12 h to reach the base of the style (Fig. 2C)

DISCUSSION

Chiococca alba presents ordered, homomorphic herkogamy, since individuals bear only one floral type, which is hermaphrodite and tubular, resulting in controlled orientation of pollinators (Webb and Lloyd 1986). Tubular flowers control the orientation of visitors' mouthparts, which are introduced to the bottom of the corolla.

Frequency of visits to flowers of *C. alba* is low when one considers flower longevity, and may be a consequence of the absence of measurable nectar, despite the presence of a basal disc similar to other Rubiaceae nectaries (Robbrecht 1988). Butterflies seem to visit *C. alba* flowers only to collect pollen, produced in large quantities, and sometimes accumulated inside the corolla tube. Despite the low frequency of visits, natural fruit set is similar to that achieved by legitimate crosses, indicating that these visits are sufficient for pollen carryover between individuals and fruit production.

Several factors contribute to the reproductive success of this species. Pollen flow of *C. alba* is maximized by the high pollen production and the low number of ovules to be fertilized. The large receptive area of the stigma and its exerted position, which make it easier to contact pollinators' body, favors pollen receipt. Finally, the relative position of male and female organs also contribute for outcrossing rates, because it may avoid interference between male and female functions, a general problem of floral architecture and a selective pressure influencing diverse floral features (Webb and Lloyd 1986). Since flowering is highly synchronized, movement of pollinators among plants is encouraged, because of the high availability of flowers, resulting in very low frequency of self-pollination (Webb and Lloyd 1986).

The herkogamy observed in *C. alba*, the pendant position of flowers and their powdery pollen are conflicting features, since self-pollen may drop onto the stigma by gravity or wind (when many grains may also be lost), or during pre-anthesis stage, germinating when the stigma becomes receptive. Thus fruit set after self-pollination is not insignificant in this species, and may contribute, together with apomixis, to the total fruit set naturally obtained (control).

The reproductive strategy of *C. alba* is different from to the expected for members of Chiococceae tribe, *e.g.*, secondary pollen presentation or distyly (Robbrecht 1988), but shows features of both strategies, herkogamous, self-incompatible flowers, similar to distylous species, and anther dehiscence in pre-anthesis buds, as frequently observed in stylar pollen presentation mechanism (Imbert and Richards 1993). The genus *Chiococca* present 43 species (Andresson 1992), and has some related genera (*sensu* Bremer 1996) showing either distyly, as *Hamelia* (Bawa and Beach 1983), or secondary pollen presentation, as *Cephalanthus* (Imbert and Richards 1993).

The occurrence of monomorphy, *e.g.*, the presence of only one floral morph, self-compatible plants in populations of typically distylous groups is interpreted as a derived character state (Hamilton 1990 and references therein). Besides presenting only pin-like flowers, *C. alba* is self-incompatible, in contrast to monomorphic populations, where self-compatibility is always recorded. Moreover, herbarium material from three other SE Brazilian states bear flowers morphologically similar to those described here. Thus we conclude that *C. alba* may present a novel reproductive mechanism which includes features of both strategies observed within the genus, e.g., distyly and pollen presentation at the style. Nevertheless, it is not possible to suggest the steps through which this new mechanism evolved.

The reproductive trends distributed among Rubiaceae tribes, as suggested by Robbrecht (1988), must be carefully considered. Distyly and secondary pollen presentation are strategies more commonly found in some tribes, however many exceptions may be found. Interestingly, almost all exceptions include only some features of the expected strategy, or even intermediate features between them. *Ferdinandusa speciosa* (Castro and Oliveira 2001) shows protandry, whereas its subtribe, Cinchoninae, includes distyly or secondary pollen presentation; *Guettarda scabra* (Richards and Koptur 1993); *Hamelia patens* (Imbert & Richards 1993); finally, *Posoqueria* spp. may present both secondary pollen presentation and distyly, whereas their tribe (Gardenieae) have exclusively the first strategy (Bawa et al. 1985).

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CONCLUSÕES

Os polinizadores das espécies estudadas estão relacionados principalmente a características florais como tamanho, cor, hora de antese e recursos disponíveis, mas parecem ser também influenciados por outros fatores, como distribuição das plantas e disponibilidade de flores. Assim, apesar das flores de todas as espécies serem tubulares e diurnas, o principal grupo de polinizadores difere entre algumas delas. *Psychotria jasminoides* e *C. alba* são polinizadas principalmente por borboletas, enquanto *P. mapourioides*, *P. pubigera* e *P. birotula* por abelhas da família e *P. nuda* por beija-flores.

As espécies estudadas podem ser divididas em três categorias, distílicas típicas (*P. jasminoides*, *P. mapourioides* e *P. birotula*), atípicas (*P. pubigera* e *P. nuda*) e hercogâmica (*C. alba*). Espécies tipicamente distílicas são aquelas que apresentam hercogamia recíproca, auto-incompatibilidade, incompatibilidade intramorfo e populações isopléticas; espécies distílicas atípicas são aquelas que não possuem uma dessas características. Apesar de utilizada aqui, a ausência de hercogamia recíproca pode não ser suficiente para que uma espécie seja considerada distílica atípica.

Todas as espécies apresentam separação espacial entre órgãos reprodutivos. *Psychotria jasminoides*, *P. birotula*, *P. mapourioides*, *P. pubigera* e *P. nuda* possuem dois morfos florais e, exceto em *P. nuda*, a hercogamia não é recíproca. *Chiococca alba* apresenta apenas um tipo de flor na população, semelhante ao longistilo. Todas as espécies são auto- e, no caso das dimórficas, intramorfo-incompatíveis e isopléticas, com exceção de *P. pubigera*, que possui mais indivíduos brevistilos.

As flores longistilas foram mais freqüentemente visitadas, o que pode indicar um fluxo assimétrico de pólen em direção a esse morfo, como é geralmente registrado para espécie distílicas e interpretado como uma conseqüência do fato do estigma das flores longistilas ser mais exposto e facilmente contatado pelos polinizadores. No entanto, uma maior freqüência de visitas não implica necessariamente em maior deposição de grãos compatíveis, nem em maior proporção de grãos compatíveis.

Apesar de cada espécie de planta distílica estar relacionada a um determinado grupo de polinizadores, as relações aqui encontradas não são especializadas, o que parece não impedir o fluxo de pólen intermorfo. Este resultado pode ser uma conseqüência da estrutura floral das flores tubulares que forçam os visitantes a introduzirem seu aparelho bucal precisamente no centro da flor, contatando os órgãos sexuais antes e após alcançarem o néctar, secretado na base do tubo. Assim, a identidade do polinizador pode não ser tão importante como geralmente sugerido para espécies distílicas. A presença de um aparelho bucal longo pode ser suficiente para alcançar os órgãos sexuais do nível inferior e manter os níveis de cruzamentos intermorfo.

A maioria das diferenças morfométricas encontradas entre os morfos corrobora a tendência dos dados registrados para espécies distílicas, destacando-se, entre elas, o maior tamanho de órgãos florais do morfo brevistilo. Em alguns casos as diferenças encontradas não eram esperadas, como o comprimento do lobo estigmático, ou eram esperadas e não foram observadas, como no caso da altura das anteras em *P. nuda*. Apesar de existirem algumas teorias que tentam explicar a funcionalidade das características secundárias, o significado adaptativo destas ainda não é totalmente compreendido.

Os dados obtidos para as espécies distílicas indicam que a hercogamia recíproca pode contribuir para as polinizações intermorfo, o nível de incompatibilidade parece ser proporcional ao equilíbrio da população e, finalmente, os desvios dos padrões morfológicos e de força da incompatibilidade ocasionalmente observados parecem não impedir a produção natural de frutos.

Chiococca alba possui uma estratégia reprodutiva distinta, que inclui características tanto da distilia (hercogamia) quanto da apresentação secundária de pólen no estilete com protandria, um mecanismo reprodutivo comumente encontrado em alguns grupos de Rubiaceae; ambos os mecanismos podem ser encontrados na subfamília Chiococceae. Apesar de possuir baixa freqüência de visitas, a produção natural de frutos desta espécie é relativamente alta, e pode incluir tanto os frutos formados por polinização cruzada quanto os obtidos por autopolinização e apomixia.