REPRODUCTIVE ECOLOGY OF *Psychotria poeppigiana* (RUBIACEAE): A COMPARATIVE ANALYSIS BETWEEN LONG-STYLED AND SHORT-STYLED PLANTS

ECOLOGÍA DE LA REPRODUCCIÓN DE *Psychotria poeppigiana* (RUBIACEAE): UN ANÁLISIS COMPARATIVO ENTRE PLANTAS LONGIESTILADAS Y BREVIESTILADAS

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ABSTRACT

We investigate morph-specific patterns in flower morphology, nectar concentration, and foraging behavior of flower visitors in a population of *Psychotria poeppigiana* (Rubiaceae), a distylous species of secondary forests in the Chocó, Colombia. The studied population had 1:1 ratio between morphs. We did not observe strict reciprocity in the position of stigma and anthers between short-styled and long-styled flowers, since the space separation between anther and stigma was greater in long-styled flowers. We did not observe differences between floral morphs in flowering and fruiting patterns. Nectar concentration was not different between floral morphs throughout the day. The relation between nectar concentration and floral visits experienced by plants of both morphs was evident. *Trigona spinipes* (bees) was the most frequent floral visitor of *P. poeppigiana*. However, *Phaethornis striigularis* (hummingbird) and *Heliconius erato venus* (butterfly) probably are more effectives as pollinators. Our results suggest that floral morphs of *P. poeppigiana* don't presented reproductive conflicts or functional gender specialization based on the manifestation of reproductive phenology, patterns of nectar concentration, and floral visits.

Key words: floral biology, heterostyly, pollination, *Pychotria poeppigiana*, rubiaceae

RESUMEN

Investigamos la morfología floral, patrones de concentración de néctar y comportamiento de forrajeo visitantes florales en una población diestílica de *Psychotria poeppigiana* en un bosques secundarios en el Chocó (Colombia). La proporción de individuos de ambos morfos florales en la población fue 1:1. La hercogamia recíproca no fue perfecta entre los morfos, ya que flores longiestiladas presentaron mayor separación espacial entre anteras y estigma que aquellas breviestiladas. Entre morfos florales no observamos diferencias en patrones de floración y fructificación, ni en concentración de azucares en el néctar en horas puntuales a lo largo del día. Es evidente la relación existente entre la concentración de néctar y las visitas florales experimentadas por las plantas de cada morfo floral. Aunque *Trigona spinipes* fue el visitante floral más frecuente en ambos morfos florales, *Phaethornis striigularis* (colibrí) y *Heliconius erato venus* (mariposa) posiblemente cumplen un rol más importante como polinizadores. Al parecer, no existen conflictos reproductivos o especialización de género entre los morfos florales de *P. poepigiana*, ya que no apreciamos diferencias en los patrones fenológicos, oferta de néctar y visitas florales entre morfos florales.

Palabras clave: biología floral, heteroestilia, polinización, *Psychotria poeppigiana*, rubiaceae

INTRODUCTION

Flowering plants or angiosperms are predominantly hermaphroditic (Bawa et al. 1985, Fetscher 2001, Barrett 2002 a, b; Cesaro *et al*. 2004), this condition where both female (pistils) and male (stamens) sexual organs are located within individual flowers can facilitate self-pollination, and cause negative consequences on fitness of species as result of self-fertilization and inbreeding (Charlesworth and Charlesworth 1987, Barrett 2002 a, b; Cesaro *et al*. 2004, Bailey and McCauley 2006). However, hermaphrodic species can reduce self-pollination since anthers and stigma can be spatially separated "herkogamy" in the flower (Lloyd and Webb 1986, Webb and Lloyd 1986, Fetscher 2001, Barrett 2002a, b; Murcia 2002).

Heterostyly is a genetically controlled floral polymorphism in which plant populations are composed of two (distyly) or three (tristyly) floral morphs that differ reciprocally (reciprocal herkogamy) in the height at which anthers and stigmas are position among cospecific flowers (Ganders 1979, Lloyd and Webb 1992, Richards and Barrett 1992, Barrett 2002a). In the case of distyly, flowers that display stigmas to height smaller to the position of anthers are called short-styled "thrum" and those with invested pattern are denominated long-styled "pin". The style-stamen polymorphism is usually accompanied by other polymorphisms (size of anthers, size and amount of pollen grain, size and shape of stigma) and a felf-incompatibility system that prevents felf- and intramorph fertilizations (Barrett and Richards 1990, Barrett 1992, Dulberger 1992, Lloyd y Webb 1992, Barrett *et al*. 2000). Thus, each floral morph produces fruits and seeds when their stigmas are pollinated with pollen of the floral morph opposed (Barrett 1992, Dulberger 1992, Teixeira and Machado 2004ab, Lopes and Buzato 2005).

The diversity of floral traits traditionally has been interpreted as "anti-selfing" mechanisms; also has been formulated that those floral traits have evolved through natural selection. However, it is likely that such traits are favored by sexual selection (Andersson 1994). The theory of sexual selection can be used to explain differences among the sexes of plants such as: major production of flowers or inflorescences (Stephenson and Bertin 1983), amount, size and shape of pollen grains (Campbell and Waser 1987, Dulberger 1992), reproductive phenology (Lloyd and Yates 1982, Stephenson and Bertin 1983), nectar production (Devlin and Stephenson 1985, Mitchell 1993, Carlson and Harms 2006), and pollinators attraction (Waser and Price 1983, Feinsinger *et al*. 1991). According to it, each floral morph can exhibit gender specialization and gain differential of reproductive success through their male and female functions (Beach and Bawa 1980, Barrett 1992, Dominguez *et al*. 1997).

In this paper, we described morph-specifics patterns in floral morphology, flowers and fruits production, nectar concentration, and foraging behavior of floral visitors in a distylous population of *P. poeppigiana* (Rubiaceae), it with the aim of to examine possible differences between floral morphs on the basis of selection sexual theory, and also, to explore the functional and adaptive significance of distyly in this species.

MATERIALS AND METHODS

Study area
We carry out this work between August 2006 and January 2007 in two fragments of forest located to right and left of the roat that of Quibdó leads to the municipality of Atrato, approximately to two kilometers of Quibdó city (5º41´ N, 76º40´ W; and 90 - 100 meters of altitude on level of sea, Pino *et al*. 2002). In Quibdó the temperature annual average is of 27ºC, and the relative humidity of 85% (Espinal 1977). Within the Biogeographic Chocó region, Quibdó belong to the Central-Norte subregion which is characterized for present the major humidity within the Biogeographic Chocó (Poveda *et al*. 2004).

Study species
Psychotria poeppigiana Mull (Art.) is a species found commonly within secondary forests, their inflorescences with red bracs and yellow flowers, and infructescences with fruit blues (when ripe) easily allows to identify this species under field conditions. *P. poeppigiana* is a distylous species with a felf-incompatibility system that prevents self- and intramorph fertilizations. Thus, only intermorph mating result in seeds developed (Coelho and Barbosa 2004, Dulberger 1992). Ripe fruits are consumed by several species of birs within the study area.

Proportion of morphs within the population and floral morphometry
We registered all the individuals in flowering

(plants of long-styled flowers and those with short-styled flowers) within the population, and thus, was determinated the ratio between morphs. On the other hand, several flowers per floral morph from ten plants of each morph were collected. Later, in each flower the follow traits were measured: corolla-tube length and diameter, anthers and stigma height (from the corolla base). Moreover, we measured the distances between anther and stigma within individual flowers (herkogamy) (Hernández and Ornelas 2003, Teixeira and Machado 2004 a, b, Consolaro *et al*. 2005, and Rossi *et al*. 2005).

Morph-specific patterns of flowers and fruits display
We select 44 plants (22 of each floral morph) at random within the population. Weekly, we registered the number of inflorescences and infructescences present in each plant (between August 2006 and January 2007). On the other hand, also were reviewed specimens of *P. poeppigiana*

deposited in the Herbario "CHOCO" of the Universidad Tecnológica del Chocó (collected within the studied area between February and July from 1976 to 2005) with the aim of to explore whether plants of *P. poeppigiana* show flowers and fruits throughout the year.

Floral biology and morph-specific patterns of nectar concentration
We registered the anthesis time (beginning and duration) of 20 floral buds, it were selected to random from ten inflorescences of ten plants of each floral morph. To the following day, the apex (bud width) in each floral bud was measured every 30 minutes throughout the day (from 0500 to 1800 h) using a digital vernier Mitutoyo CD - 8" (0.01 mm of precition). Moreover, we selected other 20 floral buds in pre-anthesis from ten inflorescences of ten plants of each floral morph. Later, these floral buds were bagged to exclude their floral visitors. We returned to flowers to the following day, and each two hours, we measured the sugars concentration
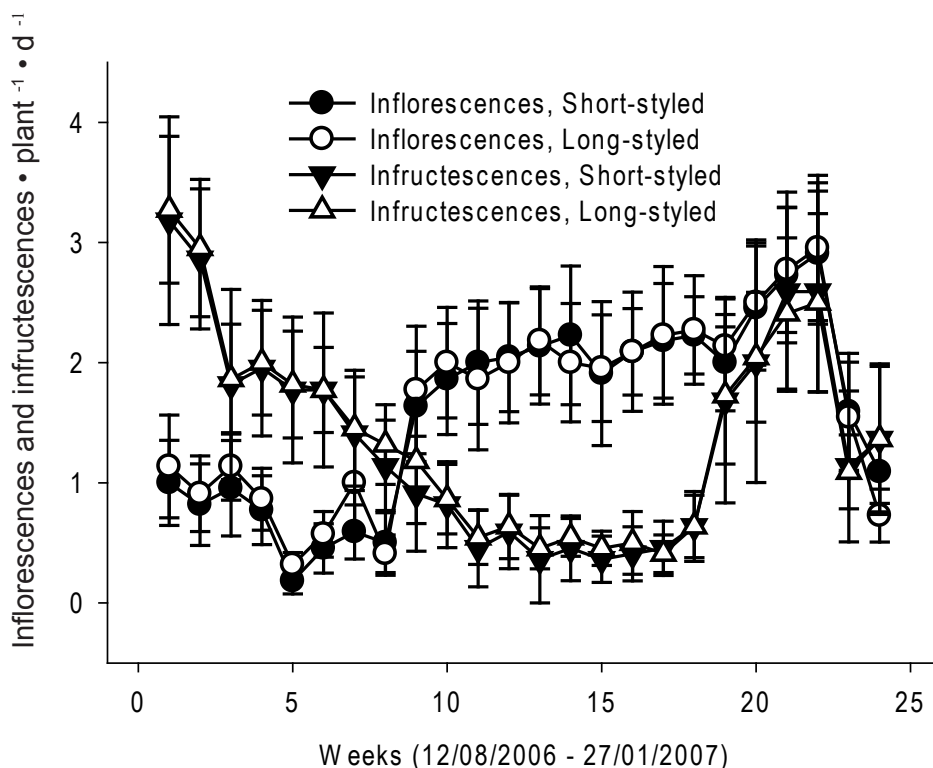

Figure 1. Temporal dynamic of inflorescences and infructescences production in long-styled and short-styled plants of *Psychotria poeppigiana* in a secondary forest (Quibdó, Chocó, Colombia). Data indicate mean ± 1 S.E; ANOVA, the floral morphs effect in both cases was P > 0.05.

Table 1. Floral measurements (Mean ± 1S.E) and results of test U Mann-Whitney for floral dimorphisms in *Psychotria poeppigiana*. ** P < 0.001; * P < 0.05; NS = Non-significant (P > 0.05).

Floral traits (cm.)	Floral morphs				U
	Short-styled	N	Long-styled	N	
Corolla-tube length	1.85 ± 0.02	20	1.83 ± 0.02	20	181.0 NS
Corrolla-tube diameter	0.63 ± 0.02	20	0.49 ± 0.02	20	65.0**
Anthers heigth	1.47 ± 0.03	20	1.07 ± 0.01	20	2.0**
Stigma heigth	0.99 ± 0.02	20	1.78 ± 0.06	20	1.0**
Stigma-anther separation	0.46 ± 0.03	20	0.63 ± 0.04	20	87.5*

using a hand refractometer (Bellingham + Stanley 45-81). The nectar concentration was measured in the following hour intervals: 0600-0800, 0900-1100, 1200-1400, 1500-1800 h. In this study, we did not recorded patterns of nectar production due to instrumental limitations for carry out that measured.

Morph-specific patterns of visits and foraging behavior of floral visitors
Through six months, we documented the dynamic of floral visits experienced by 15 plants of each floral morph. In addition, we registered the foraging behavior showed by floral visitors in plants of both morph in the following hour intervals: 0600-0800, 0900-1100, 1200-1400, 1500-1800 h

RESULTS AND DISCUSSION

Ratio of morphs within the population
We register a proportion between morphs near to 1:1 (51.4% short-styled and 48.6% long-styled; n = 105 plants). The proportion 1:1 is a condition expected in distylous populations (Barrett *et al*. 2000), it has been documented in other species of the *Psychotria genus* such as *Psychotria ipecacuanha* (Rossi et al. 2005) and *P. barbiflora* (Teixeira and Machado 2004 a). However, in some populations of distylous species, ratios different at 1:1 have been recorded; for example, Teixeira and Machado (2004 b) documented a 1:4

ratio between floral morphs of Sabicea cinerea (Rubiaceae); see also Dulberger (1992) for other cases. According to Coelho and Barbosa (2004) differences between short-styled and long-styled plants in vegetative reproductive success (clones) could alter the proportion expected between morphs of heterostylous species. We suggest that the 1:1 proportion observed in *P. poeppigiana* favor pollination success in both morph, since similar proportion between morphs within population, could facilitate that effective pollinators can transfer pollen among plants of both morphs.

Floral morphometry
Reciprocal herkogamy was evident in the population studied, but imperfect between morphs. In this since, the space separation between anthers and stigma within individual flowers was significantly larger in long-styled flowers than in those short-styled (Table 1). The reciprocal position of anthers and stigma in distylous species has been interpreted as a mechanism that promotes animal-mediated cross-pollination between floral morphs (Darwin 1877). According to this hypothesis, pollen from anthers located at different levels could adhere to different zones of the pollinator's body corresponding to the position where compatible stigmas contact the animal (Barrett and Glover 1985, Lloyd and Webb 1992, Barrett 2002 a, Ornelas *et al*. 2004 a). This way, a better precision in pollen transference is

promoted among cospecific plants, in comparison to than would occur in populations with uniform herkogamy (Barrett 2002 a). According to this, to major symmetry in the position of anthers and stigmas, a better optimization in fecundity may be expected.

Morph-specific patterns in display of flowers and fruits
The flowering and fruiting patterns ware similar between short-styled and long-styled plants. In this case, during our observations, plants of bout morphs were not different in number of inflorescences (mean ± 1S.E; short-styled = 1.60 ± 0.09 inflorescences • plant^{-1} • d^{-1}:, n = 528; long-styled = 1.64 ± 0.1 inflorescences • plant^{-1} • d^{1}, n = 528; t test = -0.30, P = 0.76) and infructescences (short-styled = 1.40 ± 0.1, n = 528 infructescences • plant^{-1} • d^{-1}; long-styled = 1.41 ± 0.1 infructescences • plant^{-1} • d^{-1}, n = 528; t = -0.29, P = 0.77; Figure 1). Equally, independently of the floral morph (P > 0.05), inflorescences and infructescences displayed in average 2.3 ± 0.1 flowers • d^{-1} and 2.0 ± 0.1 fruits • d^{-1}, respectively.

The perfect reciprocal herkogamy has been associated to better precision in pollen transference between floral morphs of heterostylous species (Darwin 1877, Lloyd and Webb 1992, Stone and Tompson 1994, Barrett 2002a, de Jong and Klinkhamer 2005). However, the similarity observed between floral morphs of *P. poeppigiana* in female reproductive success (fruit production), suggests that the disassortative pollination also can be promoted in distylous species that show imperfect reciprocal herkogamy (see Table 1). Nevertheless, it is an appreciation that should be experimentally evaluated in the future through analysis of stigmatic pollen load, pollinators behavior, and seed production in both morphs (see Ornelas *et al*. 2004 a, Hernández and Ornelas 2007, García-Robledo 2008).

On the other hand, we observed flowers and fruits in all specimens of Herbario that had been collected within the study area between February and July from 1976 to 2005, suggesting that plants of *P. poeppigiana* show flowers and fruits during whole year. However, blooming and fruiting occur of way asynchronous among individuals (pers. obs.). Extensive blooming has been interpreted as a strategy to avoid risk of
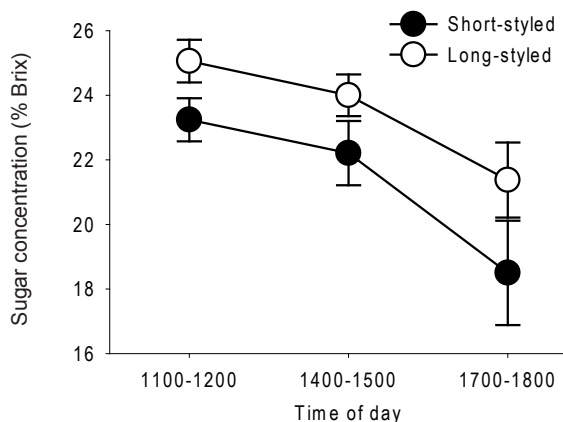

Figure 2. Temporal dynamic of the nectar sugar concentration in long-styled and short-styled flowers of *Psychotria poeppigiana* in a secondary forest (Quibdó, Chocó, Colombia). Data indicate mean ± 1 S.E; the floral morphs effect × time-of-day was P > 0.05.

blooming in unfavorable periods, thus assuring favorable periods for reproduction (Bawa 1983, Silva 1995, Coelho and Barbosa 2003).

Flowers of both floral morphs begin its anthesis between 0730 and 1200 h, and it presented longevity of 18 hours approximately. Dates liked were reported by Coelho and Barbosa (2004) in a population of *P. poeppigiana* in a Brazilian forest. Consolaro et al. (2005) suggest that a long floral longevity increases the probability of visit and cross-pollination among cospecific plants in flowering.

Nectar concentration patterns
The nectar accumulated within long-styled flowers was more concentrated than nectar of those short-styled flowers (long-styled = 23.5 ± 0.55% Brix, n = 36; short-styled = 21.3 ± 0.74% Brix, n = 36; t = -2.355, P = 0.02). However, such differences between morphs were not significant throughout the day (time-of-day effect; Mann-Whitney test; 1100-1200 h: long-styled = 25.0 ± 0.6%, n = 12; short-styled = 23.2 ± 0.6%, n = 12; U = 40.0, P = 0.06; 1400-1500 h: long-styled = 24.0 ± 0.6%, n = 12; short-styled = 22.2 ± 0.9%, n = 12; U = 49.0, P = 0.18; 1700-1800 h: long-styled = 21.3 ± 1.1%, n = 12; short-styled = 18.5 ± 1.6%; U = 46.5, P = 0.14; Figure 2).
Gender specialization based on nectar production has been documented in some dichogamous

Tabla 2. Number of visits observed (mean ± 1S.E) on plants of both floral morphs of *Psychotria poeppigiana*. ANOVA; *** P < 0.0001; NS = Non-significant (P > 0.05).

| Floral visitors | Visits · planta⁻¹ · dia⁻¹ | | | | F |
	Long-styled	N	Short-styled	N	
Amazilia tzacatl	1.1 ± 0.5	24	1.6 ± 0.3	24	3.18 NS
Eurybia lycisca	0.1 ± 0.1	24	0.1 ± 0.1	24	0.40 NS
Heliconius erato venus	0.6 ± 0.2	24	0.4 ± 0.3	24	0.30 NS
Phaetornis stringularis	1.3 ± 0.6	24	0.9 ± 0.3	24	0.10 NS
Trigona spinipes	13.5 ± 2.5	24	15.0 ± 2.9	24	0.05 NS
Trigona sp	0.8 ± 0.5	24	0.5 ± 0.4	24	1.45 NS
F	30.77 ***		29.78 ***		

species (Carlson and Harms 2006). However, in distylous species for which exist data on nectar production, floral morphs did not differ in nectar concentration and volume (*Palicourea petiolaris* and *P. fendleri*, Sobrevila *et al*. 1983; *P. demissa* (unpublished data); *Fernandusa speciosa*, Castro and Oliveira 2001; *Psychotria poepigiana*, Coelho and Barbosa 2004; *P. barbiflora*, Teixeira and Machado 2004a, b), but see exceptions in *Palicourea padifolia* (Ornelas *et al*. 2004 b) and *Fagopirum esculentum* (Cawoy *et al*. 2008).

Because sugar concentration represent the energetic value of nectar, a differential sugar concentration between morphs should not be expected (just as was observed in this work), since it could generate a disparity of floral visits and female reproductive success between them (Ornelas *et al*. 2004a, b, Manetas and Petropoulou 2002). It may explain that short-styled and long-styled plants of *P. poeppigiana* have showed similar nectar concentration patterns over time (Figure 2), and similar female reproductive success under natural conditions (Figure 1).

We observed that in both floral morphs, nectar concentration trend to low about afternoon (long-styled: 1100 - 1200h = 25.0 ± 0.6%, n = 12; 1400 - 1500h = 24,0 ± 0.6%, n = 12; 1700 - 1800h = 21.3 ± 1.1%, n = 12; Kruskal-Wallis test, X^2 = 7.17, P = 0.02; short-styled: 1100 - 1200h = 23.2

± 0.6%, n = 12; 1400 - 1500h = 22.2 ± 0.9%, n = 12; 1700 -1800h = 18.5 ± 1.6%, n = 12; X^2 = 7.19, P = 0.02; Figure 2), showing significant differences only between the morning and afternoon hours (1100-1200h vs. 1700-1800h: long-styled, U = 29.0, P = 0.01; short-styled, U = 28.0, P = 0.01; Figure 2). Similar tendencies have been documented in other distylous species such as *S. cinerea* and *P. barbiflora* (Teixeira and Machado 2004a, b). According to some researchers, the nectar concentration can vary throughout the day (Murcia 2002, McDade and Weeks 2004a), with the age of the flowers, and as result of dilution in rain's water or condensation due to evaporation (Murcia 2002). According to Opler (1983) the nectar concentration also can vary among flowers within a plant and among plant within a population.

Foraging behavior of floral visitors
The floral visitor's guild was represented by hummingbirds, butterflies, and bees. These floral visitors visited short-styled and long-styled plants about morning, but with significant differences among them, being *T. spinipes* (Himenoptera: Apidae) the most frequent floral visitor of *P. poeppigiana* (Table 2, Figure 3). Possibly, major number of visits about morning was stimulated by high levels of nectar concentration in flowers
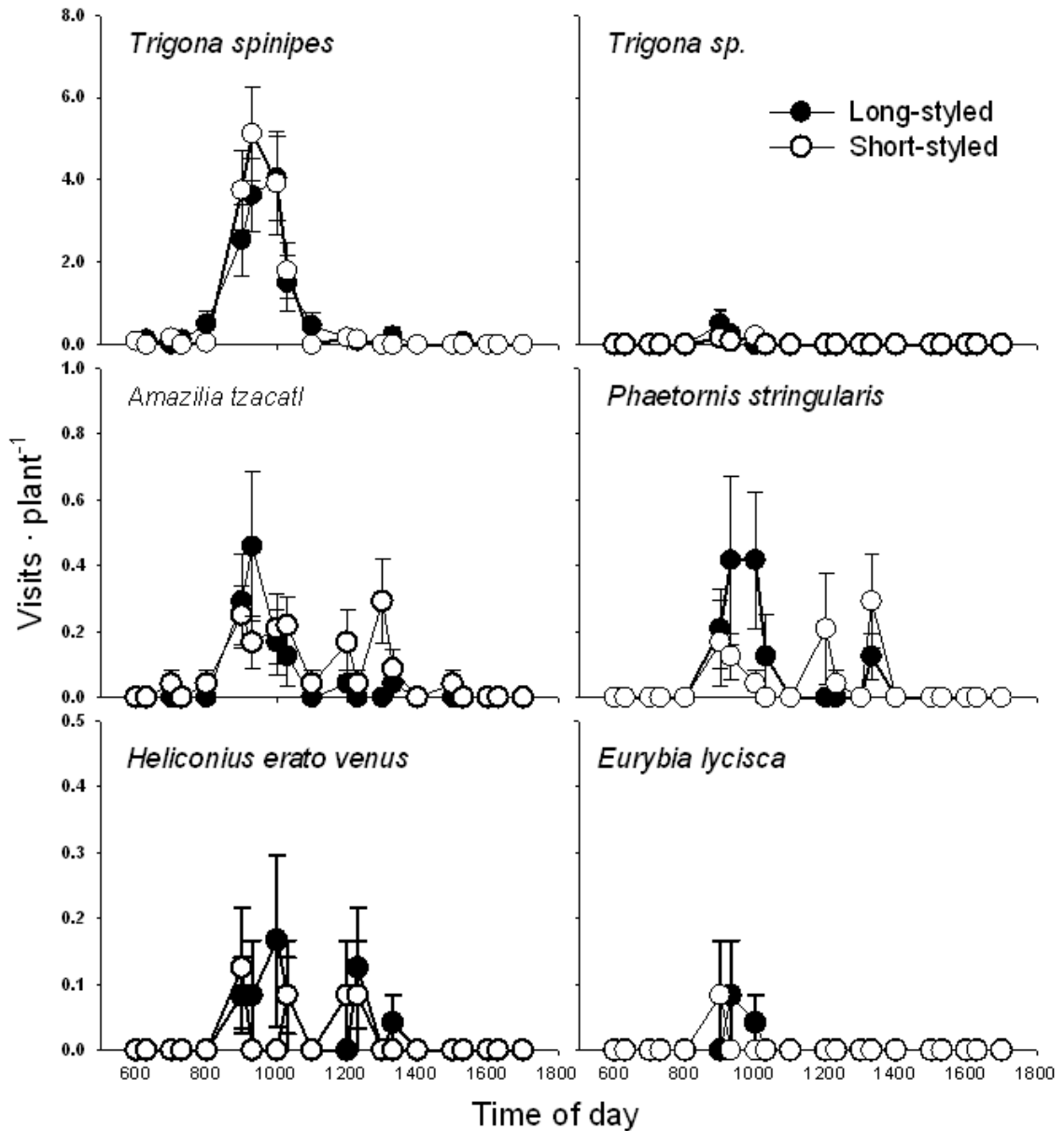
Figure 3. Flower visits on long-styled and short-styled plants of *Psychotria poeppigiana* in a secondary forest (Quibdó, Chocó, Colombia). Data indicate mean ± 1 S.E. (ANOVA; floral visitor effect, P < 0.05; the floral morph effect in all floral visitors, P > 0.05).

of both floral morphs in those hours. In some works have been documented relations between foraging behavior of the floral visitors and quality of floral nectar (see Martínez Del Rio and Eguiarte 1983, Devlin and Stephenson 1985, Ree 1997, Contreras and Ornelas 1999, Cresswell 1999, Lasso and Naranjo 2003, Ornelas *et al.* 2004 a, b).

Pollinators are subject to decisions about the time and energy that they invest for food search, that decisions are based on the perception of reward offered by flowers (Murcia 2002, de Jong and Klinkhamer 2005); thus, pollinators can change its foraging behavior whether variations in nectar levels occur (Aizen and Basilio 1998). Kearns

and Inouye (1993) argues that the visits rate of a pollinator can be affected for the quality of nectar available in flowers. Thus, nectar volume and concentration could affect reproductive success of plants (Manetas and Petropoulou 2003, Lau and Galloway 2004). According to this idea, is reasonable think that long-styled and short-styled flowers show not significant differences in nectar concentration over time (case observed in this work), since it could increase probabilities that short-styled and long-styled plants can be simultaneously visited by their pollinators over time, promoting this way, a disassortative pollen transfer within the population.

Hummingbirds

Phaethornis striigularis: This species was found foraging legitimately flowers of both morphs; also showed a non-territorial foraging behavior. Gutierrez *et al*. (2004) found that *P. striigularis* visit flowers of short corolla that often are visited by insects (just as was observed in this research). Although the visit frequency of *P. striigularis* on flowers of both morphs was lower than those of *T. spinipes* (Figure 3), we consider that this species could be an effective pollinator of P. poeppigiana, since when visiting flowers its bill can easily touch anthers and stigmas in both floral morphs, thus, pollen transference between long-styled and short-styled flowers can be realized. According to Stone (1996) the efficiency of a pollinator depends on the amount of pollen that it can remove and deposit in co-specific flowers through legitimate visits. On the other hand, Valois-Cuesta and Novoa-Sheppard (2006) suggests that a territorial behavior could represent a reproductive disadvantage on distylous species, because floral visitors restrict their feeding area to few plants, reducing this way the legitimate transference of pollen between short-styled and long-styled plants. In these cases, the majority of the effective cross-pollinations are executed by foreign visitors who can to penetrate within of the territory (Murcia 2002).

Amazilia tzacatl: We found that *A. tzacatl* visited few short-styled and long-styled plants (Figure 3), and it was observed moving aggressively to other floral visitors of its feed territories. According to Gutiérrez *et al*. (2004), *A. tzacatl* is a territorial and very aggressive hummingbird, which defends flowers of other hummingbirds, butterflies and bumblebees.

Butterflies

Euribia lycisca: This species was observed with low visits frequency throughout the study. It is a sporadic species, and likely, it is not an effective pollinator of *P. poeppigiana* since was not observed visiting both short-styled and long-styled plants.

Heliconius erato venus: This species inserted legitimately its proboscis within of short-styled and long-styled flowers to extract nectar. Although its visits frequency was few, *H. erato venus* is possibly a effective pollinator of *P. poeppigiana*, since when introducing its proboscis within flowers, pollen from anthers located at different levels could adhere to various zones of that organ, points which, corresponding to zones where compatible stigmas would contact the animal. Thus, cross-pollination between morphs would be facilitated. This idea is argued by some researchers (Barrett & Glover 1985, Lloyd & Webb 1992, Barrett 2002a).

Bees

T. spinipes: Although the visit frequency of *T. spinipes* was greater than the visit frequency of other floral visitors, its foraging behavior could be considered as antagonistic, since this species carries out perforations in the corolla-tube base for search nectar, as result of this behavior, flowers were total or partially damaged. According to Kearns and Inouye (1993), it can reduce the floral display, generating a reduction on the visits rate of effective pollinators. On the other hand, we found that *T. spinipes* when visited flowers, it did not contact stigmas of short-styled flowers. However, given the morphological adjustment between *T. spinipe* and flowers, it could be promoting pollen flow from anthers of short-styled to stigmas of long-styled flowers.

Trigona sp.: This species was observed foraging as solitary, and such as *T. spinipes*, it was found making perforations in the corolla-tube base of both morphs. According to Roubik (1989), nectar robbers removed floral nectar through holes that they open in the corolla base, being *Xylocopa* and *Trigona* bees those that frequently realize that activity. The nectar robbing can be carries out by some animals without that pollination happen (Navarro 1999).

The foraging behavior here documented for *Trigona* species has been observed in other species of Rubiaceae family such as *P. barbiflora* (Texeira and Machado 2004 a) and *Manettia cordifolia* (Consolaro *et al*. 2005). However, according to these researchers, *Trigona* bees had a neutral effect on reproductive success in these plants. Although nectar robbing can reduce the visit number of pollinators, it not always has negative effects on the fitness of the plants (Arizmendi *et al*. 1996, McDade and Weeks 2004 b).

Finally our results suggest that floral morphs of *P. poeppigiana* did not present reproductive conflicts or functional gender specialization based on the flower and fruit production, nectar concentration, and floral visitors. However, a better interpretation of it need that other aspects such as pollen deposition on stigma and seed viability be studied in this species.

ACKNOWLEDGMENTS

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