Components of Pollination Effectiveness in Psychotria suerrensis, a Tropical Distylous Shrub

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Abstract In this paper I report components of effectiveness for pollinators of a tropical distylous shrub, *Psychotria suerrensis* (Rubiaceae), which is visited by a variety of bees, wasps, and butterflies, and by two species of hummingbirds. In the field, I measured the following components of effectiveness: frequency of visits, evenness of visits across plants, and diurnal pattern of visits. I also used flight-cage experiments to compare pollen-transfer abilities of euglossine bees and heliconiid butterflies. Euglossine bees visited more frequently, visited earlier in the day, and visited a higher proportion of plants in the population than did other taxa. In flight cage experiments, bees and butterflies transferred similar amounts of pollen overall, but bees transferred significantly more inter-morph (compatible) pollen. For each component measured, euglossine bees appeared to be the most effective pollinators.

Key words Distyly · Euglossine bee · Heliconiid butterfly · Pollinator effectiveness · *Psychotria*

Introduction

Numerous studies have investigated differential effectiveness of pollinators in plants with diverse pollinator assemblages (e.g., Ornduff 1975; Primack and Silander 1975; Miller 1981; Motten et al. 1981; Tepedino 1981; Bertin 1982; Thomson et al. 1982; Motten 1983; Spears 1983; Schemske and Horvitz 1984; Montalvo and Ackerman 1986; Sugden 1986; Galen et al. 1987; Herrera 1987; Young 1988; Armbruster et al. 1989; Wolfe and Barrett 1989; Murcia 1990; Wilson and Thomson 1991; Dieringer 1992; Harder and Barrett 1993; Burd 1994). A comparison of visitor effectiveness is of interest because of the idea that the "most effective pollinator" may have an overriding selective influence on floral morphology (Stebbins 1970). In some cases, floral traits such as nectar tube depth (Robertson and Wyatt 1990), or corolla size (Galen et al. 1987) can be attributed to preferences or morphological traits of the most effective visitors. Pollinator effectiveness depends on a combination of traits, including visitation frequency and pollen-transfer capabilities (Primack and Silander 1975; Spears 1983; Schemske and Horvitz 1984; Herrera 1987; Thomson and Thomson 1992). Other components of effectiveness include the diurnal pattern of visitation in relation to floral ontogeny or in relation to other pollinators (e.g., Tepedino 1981), evenness of visitation across plants, and quality of pollen delivered (e.g., Galen and Newport 1988). Pollen quality is influenced by the proportion of intraspecific pollen, the proportion of outcross pollen, and genetic or geographic distance between donor and recipient.

For plants with physiological incompatiblity mechanisms, the compatibility reaction of pollen delivered is an important determinant of quality. For species with multi-allelic self-incompatibility systems, a large proportion of outcross pollen delivered is expected to be compatible, depending on population size and structure (de Nettancourt 1977). For distylous species, two morphs coexist in about equal frequencies, one with a long style and short stamens (pin morph), and the other with the complementary arrangement (thrum morph). In most distylous species, each morph can set seed only when pollinated by the other morph. Therefore, if pollen transfer were random in a population with equally-dispersed pin and thrum plants and equal pollen production by pins and thrums, only half of the pollen received would be compatible. A visitor that transfers abundant outcross pollen between plants will still be a relatively ineffective pollinator of a distylous species if it delivers more intra-morph than inter-morph pollen – especially when viewed in the light of male floral function.

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Pollen transfer efficiency in distylous taxa has been traditionally considered in terms of female function, or pollen receipt (e.g., Ganders 1979). But the efficiency of male floral function, or pollen donation, may also be important, especially in systems where seed set is not typically limited by pollen receipt (Lloyd and Webb 1992). When considering the effectiveness of pollinators in furthering female function, it is sufficient to compare the total numbers of compatible pollen grains received by stigmas of the two morphs. If male function of flowers is considered, however, one must examine the probability of a pollen grain being delivered to a compatible stigma versus its probability of being delivered to an incompatible stigma. These two metrics of pollination efficiency are not always equivalent (Lloyd and Webb 1992; Stone 1995); one pollinator may deposit a higher number, yet a smaller proportion, of pollen grains to compatible stigmas than another.

In this paper I report the results of a series of experiments to examine effectiveness of pollinators of a tropical rainforest shrub in the genus *Psychotria* (Rubiaceae). *Psychotria suerrensis* is visited by a diverse pollinator assemblage, including bees, butterflies, wasps, and hummingbirds. It is reported as being butterfly-pollinated (Bawa and Beach 1983), and sets seed only through legitimate (i.e., inter-morph) pollinations (Bawa and Beach 1983). I observed floral visitors in the field and measured pollen transfer by caged butterflies and bees. My observations and experiments addressed the following questions. What is the frequency of visitation by the different types of pollinators? How evenly distributed are visits across plants? Is there a difference in time of day between visits by different types of pollinators? How effective are bees and butterflies at transferring pollen between flowers of the two morphs? My measurements concern pollen transfer only; they do not extend to fruit and seed set, or to seed-siring success.

Materials and methods

Study site and species

The research was done in primary lowland rain forest at La Selva Biological Station, in Heredia Province, Costa Rica. La Selva is located at the transition between the central volcanic mountain chain and the Atlantic coastal plain and receives ≈ 4 m of rain a year, with a dry season during February and March. *Psychotria suerrensis* (J. Donnell Smith) is an understorey shrub that occurs in low elevation wet forests from Nicaragua to Panama (Taylor 1991). At La Selva, it can be found in relatively isolated populations of 50–100 individuals on level residual soils on ridgetops, separated from other populations by low-lying swamps. The population used for this study contained about 150 individuals in an area of 150×50 m, and was separated from other populations by at least 50 m. The population's location is tied into La Selva's geographical information system (GIS) data base, and locations of plants within the population are given in Stone (1994).

Individuals of *P. suerrensis* typically bear 3–15 inflorescences, each opening 0–4 flowers per day during the blooming season, which extends from mid-February to mid-March. Flowers are ≈1.4 cm long with narrow-tubular corollas and reciprocal placement of floral organs between morphs (Stone 1994). The flowers last a single day, opening just before dawn, and wilting at dusk. The time of anther dehiscence depends on relative humidity. On dry days, anthers dehisce soon after the flower opens. The flowers secrete small amounts of nectar. *P. suerrensis* has two ovules per flower, and one or two seeds per fruit. It sets seed only when pollinated by the opposite morph (Bawa and Beach 1983). Pollen production does not differ by morph (Stone 1994).

Pollinator observations

I identified focal plants as those having more than 20 inflorescences. Casual observation had led me to believe that plants with large displays had higher visitation rates than plants with smaller displays, and I wanted to make as many observations as possible in the available time. Thus, visitation rates reflect those of the most attractive plants in a population. Of the 12 focal plants, 11 were in the understorey of primary forest. The other plant was at the edge of a recent treefall gap, about 300 m distant from the understorey population. This individual continued flowering for another week after other plants had finished, allowing me to collect more observations on it than on any of the understorey shrubs. I did not collect voucher specimens of insects; visits were infrequent enough that collecting might have affected pollination patterns. Visitors were therefore classified according to easily identifiable groups, rather than by species. I was able to collect visitors in other populations of *P. suerrensis* nearby; these were identified by D. Roubik and are deposited at INBIO, in Costa Rica.

I spent a total of 40 h over 19 days observing pollinator visits across 12 focal plants during the 1992 flowering season. I stationed myself at each focal plant for at least 0.5 h and recorded arrival and departure time and identity of floral visitors. For a statistical comparison of visitation rates across taxonomic groups, I used day as the replicate unit. Visits/hour were compared using a Kruskal-Wallis test. Evenness was calculated as the number of focal plants on which a type of visitor was seen, divided by the total number of visits by that taxon. *G*-tests were used to compare pollinator classes in terms of their diurnal visitation patterns and visitation to understory plants versus the plant at the edge of the treefall gap.

Flight cage experiments

Because pollinator effectiveness in distylous species depends on the amount and proportion of legitimate pollen transfer, I designed flight cage experiments to measure the efficiencies of two contrasting pollinators at four transfer types: pin to pin, pin to thrum, thrum to pin, and thrum to thrum. All recipient flowers were emasculated, so that self-pollen deposition would not obscure patterns of pollen transfer. Two-way analysis of variance was then used to examine (1) overall levels of pollen transfer by each pollinator, and (2) the interaction of pollinator species by pollen-transfer category (legitimate vs. illegitimate). Quantity of pollen transferred was revealed by the first comparison. The second comparison indicated whether pollinators transfer the same proportion of legitimate grains; i.e., whether the male function of distyly is served equally by both. The same data set analyzed by *t*-tests allowed comparisons of pollinators in terms of fulfilling legitimate pollen delivery, or female function. Finally, chi-square tests contrasted the total number of pollen grains transferred in all four transfer types to the total number expected if transfer were random.

Butterflies

In 1993, I conducted flight cage experiments with the non-pollen feeding heliconiid butterfly *Dryas julia* (Nymphalidae). Although this species does not visit *P. suerrensis*, it performs well in flight cages, in contrast to the skippers, ithomiid, and riodiniid butterflies seen in the field. Both *D. julia* and the natural pollinators have proboscides that are much longer than the corolla tube. This is important because, for butterflies other than pollen-feeding heliconiids (Gilbert 1972), pollen carried in the facial cavity tends to vastly exceed the amount transported by the proboscis (Courtney et al. 1982; Jennersten 1984). *D. julia* also shares with the natural lepidopteran pollinators (and in contrast to bees and hummingbirds) the habit of coiling its proboscis between visits, re-distributing pollen originally picked up on various parts of the proboscis (J. Stone, unpublished work). The use of *D. julia* as a model system may overestimate per-visit effectiveness by butterflies, however, since it has been found that nymphalid butterflies tend to transport more pollen grains (Jennersten 1984) and effect more pollinations (Herrera 1987) than do butterflies from other taxonomic groups.

I purchased 20 pupae from Finca Mariposa and 10 pupae from Rara Avis, both in the Atlantic lowlands of Costa Rica. When the adults emerged, I marked their wings and placed two to three each in small flight cages (about 20 cm diameter × 30 cm high). To train each butterfly to feed on *P. suerrensis*, I unrolled its tongue and inserted the tip into a corolla enriched with 30% honey solution. Following the training period, butterflies were allowed to fly and feed freely within their cages. Experimental trials of pollen transfer were conducted at 0930–1030 hours using hungry butterflies. The amount of nectar naturally available in *P. suerrensis* flowers visited during experimental trials was insufficient to keep butterflies alive, so I placed feeders in each cage after the butterflies had finished their pollen transfer trials. A feeder consisted of an inflorescence of *P. suerrensis* in a tube of water. At 1200 hours and 1550 hours I used an electronic pipette to fill the corollas of the flowers in the inflorescence with 30% honey solution.

For experimental trials, I took cuttings of *P. suerrensis* and placed them in test tubes containing water and Floralife (Burr Ridge, Illinois). Cuttings continued to open normal-looking flowers for 3 days, after which they were discarded. Between 0530 and 0600 hours, when the corollas had just opened, I removed anthers from flowers designated to be recipients. To conduct a trial, I presented a clean, free-flying butterfly with two flowers of the donor morph followed by an emasculated recipient flower of the opposite morph. I then presented it with two more donor flowers followed by an emasculated recipient of the same morph as the first recipient. That butterfly was not used again that day. Note that the design is intended to be conservative in terms of measuring inter-morph pollination efficiency; any pollen carryover will accrue to intra-morph pollinations. I alternated between trials using the two morphs as recipient types, for a total of 25 trials of each type. Immediately after each trial, I placed pistils of the recipient flowers in clean labeled microcentrifuge tubes. Between 1200 and 1400 hours, I mounted the stigmas in fuchsin jelly (Kearns and Inouye 1993) on consecutively numbered microscope slides.

Bees

In 1994, I conducted flight cage experiments with male *Euglossa tridentata*. Euglossines were hard to catch at flowers because of their speed and low visitation rates. Therefore, I caught bees by brushing a piece of filter paper with eugenol and suspending it on the edge of primary forest, near a population of blooming *P. suerrensis*, and netting males attracted by the odor. The extended tongue of *E. tridentata* measured about 1 cm long, whereas the tongues of *Euglossa* species caught at flowers (*E. gorgonensis* and *E. erythrochlora*) were about 1.2 cm long. I kept three bees at a time in a 50 × 50 × 100 cm flight cage, using a total of 15 bees during the course of the experiment. I trained the bees to feed in the flowers by presenting hungry bees with flowers supplemented with 50% honey solution. Trials were conducted at 0900–1100 hours, then flowers with supplemental "nectar" were placed in the flight cage at 1200 hours.

To conduct a trial, I presented a bee with a single donor flower followed by three emasculated recipients in one of four sequences: pin pin pin thrum, pin thrum thrum thrum, thrum pin pin pin, or thrum thrum thrum pin. The change in design from the 1993 experiment was intended to serve two functions. First, I could reduce the amount of variability in the pollen-transfer data by averaging the stigmatic pollen loads of the first two recipients. Second, visits to three recipients would reduce the amount of pollen carried over to subsequent trials. Each bee performed up to four sets of trials each day, with at least 1 min between trials. I conducted 30 trials for each combination of morphs.

Flight cage experiments: contamination by self pollen

Despite efforts to emasculate flowers as soon as the corollas were opened, some self pollen was deposited on stigmas from anthers that dehisced in bud. This problem was more serious for thrum flowers, whose high-level anthers experienced lower relative humidity than did the low-level anthers of pins, and more serious in 1993, when the dry season was unusually severe. A slight pollen dimorphism in this species allowed me to address the contamination problem; although the size distributions of the two morphs overlap, they are statistically distinguishable (Stone 1994). Mean size for pins is 39.6 μm (SE=0.21; n=33 individuals) and mean size for thrums is 47.4 μm (SE=0.41; n=32 individuals; $P<0.001$, *t*-test).

The level of contamination on a recipient stigma should be independent of the morph of the donor flower. I used this as a basis to adjust for contamination as follows. I measured ten pollen grains on stigmas from each of six pure pollen loads of each morph, using an ocular reticule at 400×. Using this distribution of pollen grain sizes, I established a size cutoff point above which most grains would be thrums and below which most would be pins. I then counted all of the inter-morph transfers, dividing pollen loads into pin and thrum based on the cutoff I had established. Pollen grains in the recipient size class were considered contaminants. The overlapping size distributions of the two types of pollen grains led to unavoidable misidentification of morph of some grains. To estimate the degree of misidentification, I calculated the area of a normal distribution that fell beyond the cutoff point that I had established (Rohlf and Sokal 1969). Pin grains varied less in size than thrums. On average, 7.8% of pin grains were misidentified as thrums, and 21.5% of thrum grains were misidentified as pins. For each recipient type, I generated two simultaneous equations with two unknowns: the actual degree of transfer, and the degree of contamination by self-pollen (Appendix). The equations use the initial estimates of contamination and transfer, along with the proportion of misidentified grains of each morph, to solve for the actual number of contaminant and transferred grains. I then inserted the initial transfer and contamination estimates from each inter-morph load into the simultaneous equations to solve for the adjusted estimate.

The resulting transfer estimates were prepared as follows for the analysis of variance. For the 1994 data, I used the mean stigmatic pollen load of the two recipient flowers. For intra-morph transfers in both years, I subtracted the median contamination level for that recipient morph for that year and then changed any negative values to zero. The adjusted counts were square-root transformed to meet assumptions for analysis of variance. I used SYSTAT (Wilkinson 1992) to conduct a two-way analysis of variance with fixed factors of pollinator (butterfly vs. bee) and transfer type (pin to pin, pin to thrum, thrum to pin, and thrum to thrum). Residuals were normally distributed (Kolmogorov-Smirnov test $P>0.10$).

Pollinator effectiveness

Models seeking to quantify pollinator effectiveness are typically multiplicative functions of those components deemed most important for the pollination system at hand (e.g., Thomson et al. 1982; Lindsey 1984; Sugden 1986; Armbruster et al. 1989). The model presented here follows the same format, but includes several components not usually considered.

Let $E=f\,e\,d\,t\,q$, where:

E=pollinator efficiency,
f =relative frequency of visits (visits/h of taxon, as proportion of all visits/h),
e =evenness of visitation (number of plants visited/number of visits by taxon),
d=diurnal pattern of visitation (proportion of visits before pollen depleted),
t =mean of square-root transformed pollen grains transferred per visit, and
q=relative pollen quality (in this case proportion of compatible pollen).

Diurnal pattern for this study is divided into two categories: before or after 0900 hours. On two mornings, I monitored pollen availability on several large shrubs, and found it to be depleted by that time. As written here, all independent variables range from 0 to 1, with the exception of t. To standardize E so that it would be comparable across study systems, E could be divided by the mean amount of pollen transferred per visit across all taxa. The resulting ratio would range from 0 to 1. Alternatively, E could be divided by the number of ovules, so that comparisons across systems would give information about pollen deposited per ovule.

Results

P. suerrensis was visited by a wide variety of pollinators, including hummingbirds, euglossine and other bees, and butterflies (Table 1). The most frequent visitors were hymenopterans. One species, *Trigona fulviventris*, was practically ubiquitous. Individuals had extremely long residence times at single plants. The mean of 7.2 min that I was able to observe (n=38, SE=1.15) is almost certainly an underestimate, since it was not always possible to follow bees when they traveled between inflorescences on an individual plant. *Trigona* foraged for both pollen and nectar. To feed on nectar, they chewed holes in corolla bases, and so did not contact anthers or stigma. Their presumably low rates of inter-plant movement (based on long residence times) combined with their

Table 1 Floral visitors observed at *Psychotria suerrensis* at La Selva, Costa Rica

Hummingbirds:	
Klais guimeti	(Violet-headed hummingbird)
Phaethornis longuemareus	(Little hermit)
Thalurania colombica	(Crowned woodnymph)
Hymenoptera:	
Euglossa erythrochlora	(Euglossini; Apidae)
Euglossa gorgonensis	
Exaerete frontalis	(Euglossini; Apidae)
Neocorynura sp.	(Augochlorini; Halictidae)
Paratetrapedia lugubris	(Exomalopsini; Anthophoridae)
Thygater analis	(Eucerini; Anthophoridae)
Trigona fulviventris	(Trigonini; Apidae)
various unidentified bees and wasps	
Butterflies:	
Aeria eurimedia	(Ithomiinae; Nymphalidae)
Parides childrenae	(Papilioninae; Papilionidae)
various unidentified	(Riodinidae and Hesperiidae)

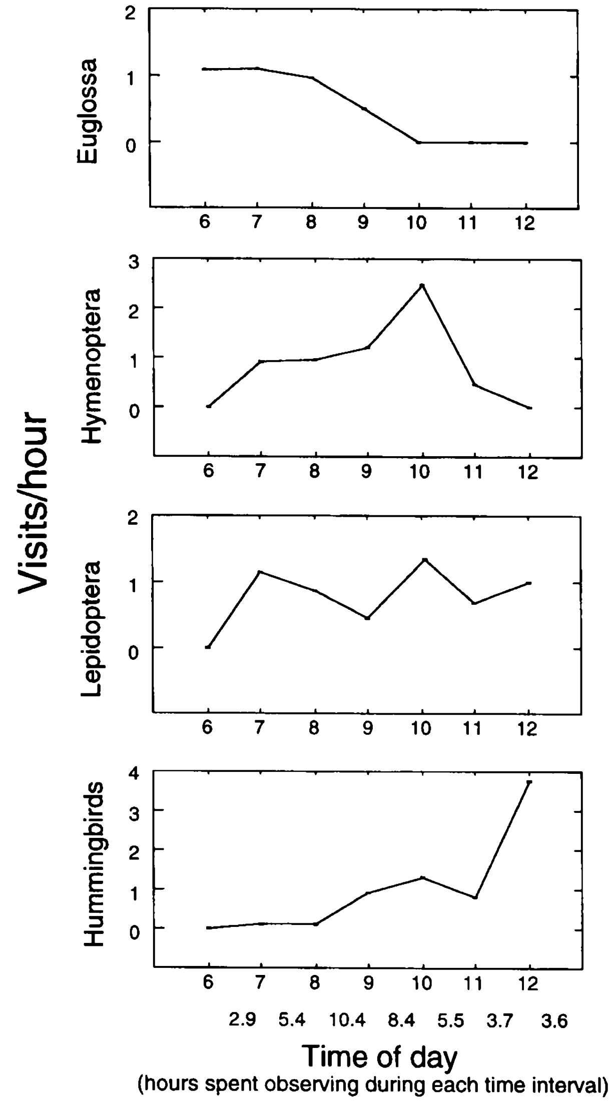

Fig. 1 Number of visits per hour by categories of floral visitors to *Psychotria suerrensis*. The value shown for each time of day is the mean for the hour beginning at that time, except for the values shown for 1200 hours, which include observations until 1700 hours. "Hymenoptera" includes bees and wasps other than *Euglossa* and *Trigona*

Table 2 Visitation rates and evenness of visitation for the four major taxonomic categories. *Trigona fulviventris* were frequent visitors but I do not consider them pollinators, so they are not included with other Hymenoptera. Differences in visitation rates are not statistically significant

Taxon	Visitation rate (visits/hour)	Evenness of visitation (number of individual plants visited/number of visits)
Euglossa spp.	0.55	0.41
Other Hymenoptera	1.02	0.17
Butterflies	0.80	0.16
Hummingbirds	0.55	0.09

Table 3 Log-linear model with *a posteriori* contrasts for pollinator categories visiting focal plants at 3 time intervals: 0600–0759, 0800–0959, and 1000–1700 hours. Significant *P* values indicate that the group is heterogeneous with respect to the time of day of observed visits; *df*=6 for all contrasts

Group	*G*	*P*
All	19.4	0.01
Other Hymen. + butterflies	1.3	0.975
Other Hymen. + butterflies + Euglossa	19.9	0.01
Other Hymen. + butterflies + birds	10.2	0.5

Table 4 Number of visits by taxon groups to *Psychotria suerrensis* in the understory and at the edge of a treefall gap. Values for *a posteriori* log-linear contrasts are shown below (*df*=3 for all contrasts). Significant *P* values indicate that the group is heterogeneous with respect to its visitation rates to focal plants in the understory versus the gap edge

Taxon	Shade	Sun
Euglossa spp.	22	0
Other Hymenoptera	26	15
Butterflies	18	14
Hummingbirds	1	21

Group	*G*	*P*
All	53.9	0.000
Other Hymen. + butterflies	0.4	0.995
Other Hymen. + butterflies + *Euglossa*	19.2	0.000
Other Hymen. + butterflies + birds;	25.6	0.000

Table 5 Square-root transformed mean stigmatic pollen loads with their associated standard errors, and Model I analysis of variance for euglossine bees and heliconiid butterflies transferring pollen between all combinations of morphs in a distylous *Psychotria*. Means are corrected for contamination by self-pollen and for misidentification of pollen morph (see text)

	Bees Mean	(*n*, SE)	Butterflies Mean	(*n*, SE)
All transfers	3.6	(116, 0.25)	3.6	(82, 0.30)
Pin to pin	2.5	(25, 0.53)	3.4	(24, 0.54)
Pin to thrum	3.8	(31, 0.48)	2.9	(16, 0.66)
Thrum to pin	4.0	(28, 0.50)	2.3	(20, 0.64)
Thrum to thrum	4.1	(32, 0.47)	6.0	(25, 0.54)
Mean sqare-root transformed contamination by self-pollen:				
To pins	1.6	(51, 0.22)	1.3	(20, 0.36)
To thrums	2.7	(61, 0.25)	7.2	(19, 1.15)

Source	SS	*df*	MS	*F*	*P*
Transfer type	149.51	3	49.84	6.90	0.000
Pollinator	0.25	1	0.25	0.00	0.953
Interaction	103.53	3	34.51	4.77	0.003
Error	1373.40	190	7.23		

habit of robbing nectar make it unlikely that they are effective pollinators (Roubik 1982; on *Psychotria*, Roubik 1989, p. 156). In addition, single emasculated flowers from two shrubs of each morph of *P. suerrensis* visited by only *Trigona* had no pollen deposited on their stigmas (n=4). Visitation rates of *T. fulviventris* are excluded from subsequent analyses.

Among the remaining bees and wasps, *Euglossa* was the single most frequent genus represented. It comprised about one-third of all Hymenoptera seen on focal plants (Table 2). Both male and female *Euglossa* visited *P. suerrensis*. Although it was not possible to identify the sex of the bees while they were visiting, it is likely that the early-morning visitors were mostly females, since, at least in flight cages, males are inactive in the early morning. Females collect pollen and forage for nectar. Visitation rates for the four taxonomic categories (*Euglossa*, other hymenopterans, butterflies, and hummingbirds) were not statistically different (Kruskal-Wallis H=7.46, n=19, df=3, P<0.06), although the trend suggests that "other hymenopterans" may have higher visitation rates than *Euglossa* or hummingbirds (Table 2).

Classes of visitors differed in the evenness of their visitation rates across plants (Table 2). A goodness-of-fit test of the observed number of plants visited by members of the four groups to the null hypothesis of equal expectation shows a highly significant departure from equal evenness (G=27.96, df=2, P<0.001; Sokal and Rohlf 1981). *Euglossa* spp. visits were the most evenly distributed; the 22 sightings occurred across nine focal plants. Other Hymenoptera and butterflies had intermediate levels of evenness; their visits were spread across about half of the focal plants. Hummingbirds had the least even distributions, being seen at only 2 of the 12 focal individuals. Classes of visitors also differed in the time of day of their visits (Fig. 1; Table 3). Hummingbirds tended to visit later in the day than other taxa, although this difference was not statistically significant, probably because of pooling of time intervals. *Euglossa* spp. visited significantly earlier in the day than other groups of floral visitors (Fig. 1; Table 3).

The plant at the edge of the treefall gap experienced a different spectrum of visitors than the plants in the understorey (Table 4). It received all but one of the hummingbird visits to focal plants and no visits by *Euglossa*. The frequency of visits by butterflies and Hymenoptera taken as groups did not differ between the two habitats; however, this may be because distinct taxa are lumped into broad classes. Field descriptions of visitors support this possibility; for example, *Exaerete* was seen twice visiting the plant on the edge of the gap and never on any understorey shrubs.

In the flight cage experiments, total amount of pollen transferred across all four combinations of morphs did not differ between bees and butterflies (Table 5). Butterflies transferred an average of 13.7 pollen grains, and bees transferred an average of 13.1 grains. Although these numbers are not different, bees may transfer pollen at a higher rate overall, since butterflies in the experi-

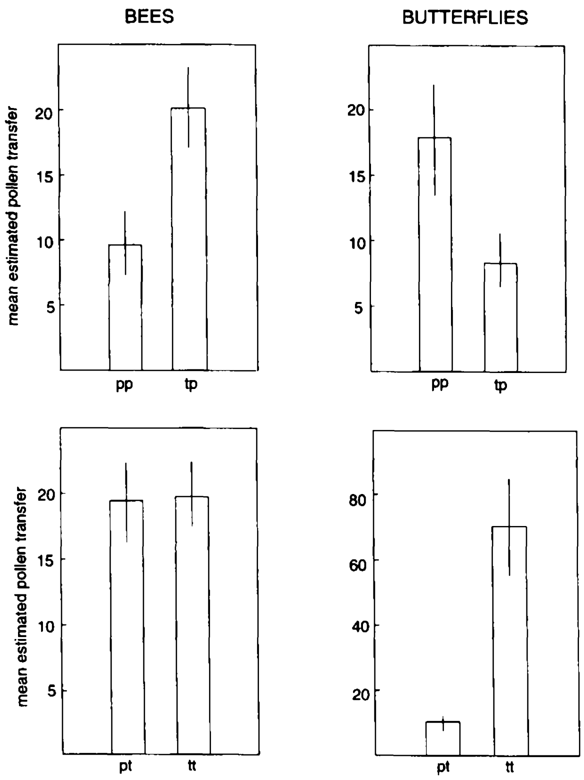

Fig. 2 Mean stigmatic pollen loads, corrected for self-pollen contamination and mis-identification of morph, following bee and butterfly visits to all combinations of donor and recipient morphs. The *first letter* under each *bar* indicates the donor morph (pin or thrum), and the *second letter* the recipient morph. *Vertical bars* are standard errors. Note change in scale for *lower right panel*. Means are not equal to the square of the values presented in Table 5, since the mean of the square roots is not equivalent to the square of the mean square roots

ments visited two donor flowers but bees visited only one. A more important consideration for distylous species is the relative rates of legitimate, or inter-morph transfer. Considering female function only, there was a trend for bees to deliver more compatible grains to thrums than did butterflies (Table 5; Fig. 2), but this difference is not statistically significant (t-test with pooled variances on square-root transformed variates, T=1.70, df=46, P=0.096). For pins as recipients, bees delivered significantly more compatible grains than did butterflies (Table 5; Fig. 2; T=3.40, df=40.1, P=0.002).

In order to consider the effectiveness of pollinators from the perspective of male floral function, we need to examine the proportion of pollen deposited that is compatible. The significant interaction between transfer type and pollinator type confirms what the means suggest: bees effected more pollen donation to compatible stigmas than did butterflies (Table 5, Fig. 2). The interaction is not due simply to the very high rates of thrum to thrum transfer by butterflies. When pins alone are considered as recipients, the transfer type by pollinator type interaction is still significant (P=0.011). With thrums alone as recipients, the interaction is also significant, at P=0.017.

Table 6 Components of effectiveness and overall effectiveness of taxa visiting *Psychotria suerrensis*. E effectiveness, f relative frequency of visitation, e evenness, d diurnal pattern, t pollen transfer, q pollen quality). No values of t or q are available for hummingbirds or for Hymenoptera other than *Euglossa*; therefore values for *Euglossa* were substituted to permit a comparison of effectiveness based on other components. *Trigona fulviventris* were frequent visitors but are not included here, since they appear to be ineffective pollinators

Taxon	f	e	d	t	q	E
Euglossa	0.188	0.41	0.86	3.62	0.56	0.134
Butterflies	0.274	0.16	0.37	3.70	0.39	0.023
Birds	0.188	0.09	0.03	–	–	0.001
Hymenoptera	0.349	0.17	0.31	–	–	0.036

Another way of reporting pollen transfer effectiveness is to consider the departure from random pollen transfer. For bees, pollen transfer was significantly non-random (χ^2=112.96, P<0.001), mostly due to a deficiency of pin to pin transfers. For butterflies, pollen transfer was also significantly non-random (χ^2=2023.18, P<0.001), mostly due to an excess of thrum to thrum transfers. Whereas bees delivered pollen from pins more efficiently than at random, butterflies delivered pollen from thrums less efficiently than at random. For fulfillment of both male and female fuction, as represented by pollen donation and receipt, the performance of bees equalled or surpassed that of butterflies.

When taken together, the components listed above indicate that bees in the genus *Euglossa* are the most effective pollinators of *P. suerrensis* (Table 6). They visit early in the morning, when pollen is most likely to be available. They are frequent visitors, exceeded in frequency and in evenness of visitation only by the presumably parasitic *Trigona fulviventris*. Based on flight-cage experiments, their delivery of compatible pollen equals or exceeds delivery levels for butterflies. Finally, legitimate pollen donation by bees surpasses legitimate donation by butterflies: for pins as donors, bees performed better than random legitimate transfer, whereas for thrums as donors, butterflies performed poorer than random legitimate transfer.

Discussion

Components of effectiveness

The most effective pollinators are those that cause the greatest number of seeds to be set or sired. Pollinator effectiveness is difficult to measure directly, so many workers have instead reported components of pollinator effectiveness (but see Motten et al. 1981; Tepedino 1981; Motten 1983; Spears 1983; Schemske and Horvitz 1984; Herrera 1987; Young 1988). Visitation rate is the easiest component to measure, but perhaps the least informative. It is obvious that an effective pollinator must have a nonzero visitation rate, but visitation rate is a rather poor predictor of pollination effectiveness, since it cannot distinguish between good pollinators and nectar or pollen thieves. For example, *Trigona fulviventris* would have emerged as the dominant pollinator in this study if visitation rates alone were considered.

Evenness of visitation among plants adds valuable information (Herrera 1988). An individual pollinator who visits many plants in a population will be a more effective pollinator than one who periodically returns to a single plant. Evenness of visitation as measured here can not evaluate the movements of individual pollinators, but it does exclude classes of visitors, such as the hummingbirds in this case, that are only seen on one or a few individual plants. Two types of feeding patterns could cause uneven visitation across plants. Some small insects, such as *T. fulviventris* and the butterfly *Aeria eurimedia*, may be able to meet their energetic requirements by taking nectar only from the flowers of an individual plant. Hummingbirds, in contrast, may visit scattered individuals of one species simply because they lie along a line founded upon individuals of a more rewarding species (Feinsinger and Chaplin 1975). For example, Little Hermit foraging trips to *P. suerrensis* usually included visits to neighboring *P.* (=*Cephaelis*) *elata* and *P.* (=*Cephaelis*) *glomerulata*. Whether visitors have long residence times or visit only scattered individuals, they are less effective than if they moved between many individuals of a single species.

A third component of effectiveness is diurnal visitation pattern. Time of visitation relative to dehiscence schedules has obvious import for pollination effectiveness (Tepedino 1981; Murcia 1990; Spears 1983; Thomson and Thomson 1992; Ashman et al. 1993). In plant species with simultaneous anther dehiscence, large proportions of pollen are removed from the anthers by the first few visits (Thomson and Thomson 1989; Harder 1990; Young and Stanton 1990; Wilson and Thomson 1991; Harder and Barrett 1993). Further visits may enhance female function, but only if stigmas are not yet saturated and pollen reservoirs are not exhausted throughout the population. Additionally, the first pollen grains to reach a stigma may sire the most seeds, so the pollinators whose visits most nearly track anther dehiscence will be the most effective. Euglossine bees were the only visitors in this population that performed the majority of their visits before pollen was removed from the anthers.

The next component of pollinator effectiveness measured here, pollen-transfer ability, can supersede all components relating to visitation patterns. A visitor may have well-timed, frequent, and evenly distributed visits but still perform poorly if it transfers few pollen grains between plants. Pollen-transfer ability commonly varies widely between floral visitors (e.g., Ornduff 1975; Primack and Silander 1975; Bertin 1982; Herrera 1987; Wilson and Thomson 1991), but not always (Motten 1983; Pettersson 1991). In some cases, species that cause the highest pollen transfer or the highest seed set per visit have lower visitation rates than less efficient pollinators (e.g., Schemske and Horvitz 1984). Both butterflies and bees in flight cage experiments transferred pollen grains at about 6 times the number of ovules on a per-visit basis. It is likely that hummingbirds would also have good per-visit transfer rates, based on their performance on *Palicourea lassiorachis*, a rubiaceous species with similar floral morphology (Feinsinger and Busby 1987).

The final component, quality of pollen, encompasses a variety of attributes. Vectors may differ in the proportion of self versus outcross pollen (Arnold 1982; Galen and Newport 1988), the proportion of inter-specific pollen deposited (Galen and Newport 1988), or the geographical distance separating pollen donors from recipients (Waser 1982; Murawski and Gilbert 1986; Herrera 1987). In self-incompatible species, the single most important determinant of quality is compatibility. The proportion of compatible grains would be difficult to determine in species with monomorphic incompatibility, but is expected to be high unless the population is structured according to compatibility type or has a depauperate number of S-alleles (Byers and Meagher 1992; Reinartz and Les 1994). In distylous species, in contrast, the null expectation of compatible pollen transfer should be less than half, depending on assumptions about pollen carry-over and the sequence of visits to flowers of the two morphs. Pollinators commonly visit numerous flowers on an individual plant, and would be unlikely to distribute as much intermorph pollen to the later flowers in the sequence as to the earlier ones. In addition, pollinators will often visit individuals of the same morph on consecutive visits. Measurements in flight cages between alternating morphs represent the ideal condition, and presumably overestimate the true amount of intermorph transfer for both types of visitors.

In distylous species, evaluation of quality of pollen transfer differs depending on whether the focus is on the efficiency of pollen donation or pollen receipt. Even a pollinator who disperses pollen at random could be successful at depositing large numbers of compatible pollen grains, if its overall transfer level were high enough. For example, if we imagined that butterflies transferred 10 times more pollen overall than the measured amounts, they would be superior to bees in terms of delivering compatible pollen to stigmas. Effectiveness of pollen receipt is strongly influenced by overall transfer levels. In contrast, for efficient fulfillment of male function, a pollinator must transfer intermorph pollen grains with a higher probability than it transfers intramorph grains. This component of quality is independent of overall quantity delivered. In the case of *P. suerrensis*, bees transferred pollen produced by pins to compatible stigmas at a better than random rate. Butterflies, in contrast, transferred pollen produced by thrums to compatible stigmas at a poorer than random rate. Efficiency of pollen donation is expected to be especially important in systems where seed set is typically not limited by pollen receipt (Lloyd and Webb 1992), but is important even when seed set is pollen-limited.

Do the components yield the whole?

Measuring components of effectiveness has practical advantages over attempting to measure effectiveness directly, especially in systems with low visitation rates. Measurement of components can also yield information about the mechanisms whereby a particular visitor exerts high effectiveness. Integrating the components into an overall measurement of performance is problematic, however. Although the multiplicative model is the most common approach to combining components of effectiveness, it has the obvious drawback that the weighting of the different terms is completely arbitrary. For example, high frequency of visitation could, in fact, be less important than pollen-transfer capability. In addition, factors such as diurnal pattern or evenness might be so important that they make other factors irrelevant. In this case, the components for *Euglossa* are consistently equal to, or higher than, components for other taxonomic groups. However, for situations where components give contradictory information, care must be taken to evaluate their relative importance in an overall assessment of effectiveness.

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Appendix

Derivation of simultaneous equations for correcting pollen-transfer estimates when overlap occurs between the size distributions of pollen from the two morphs

7.8% of pins are mis-identified as thrums (fall into the thrum size class)

21.5% of thrums are mis-identified as pins (fall into the pin size class)

For thrum to pin transfers:

initial thrum count=actual transfer – (0.215)(actual transfer)
+ (0.078)(actual contamination), and

initial pin count=actual contamination – (0.078)
(actual contamination) + (0.215) (actual transfer)

Simplifying both equations:

initial thrum count=0.785 (actual transfer)
+ 0.078 (actual contamination)

initial pin count=0.215 (actual transfer)
+ 0.922 (actual contamination)

To find actual transfer and actual contamination, insert initial counts and solve both equations simultaneously.

For pin to thrum transfers:

initial pin count=actual transfer – (0.078)(actual transfer)
+ (0.215)(actual contamination), and

initial thrum count=actual contam – (0.215)(actual contam)
+ (0.078) (actual transfer)

Simplifying both equations:

initial pin count=0.922 (actual transfer)
+ 0.215 (actual contamination)

initial thrum count=0.078 (actual transfer)
+ 0.785 (actual contamination)

To find actual transfer and actual contamination, insert initial counts and solve both equations simultaneously.

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