

## Pollinator visitation in populations of tristylous *Eichhornia paniculata* in northeastern Brazil

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Received November 29, 1990 / Accepted in revised form September 20, 1991

**Summary.** The frequencies of floral morphs in populations of tristylous *Eichhornia paniculata* often deviate from the theoretical expectation of equality. This variation is associated with the breakdown of tristily and the evolution of self-fertilization. Differences in morph frequencies could result from selection pressures due to variable levels of insect visitation to populations and contrasting foraging behavior among the floral morphs. We estimated pollinator densities in 16 populations and quantified visitation sequences to morphs in five populations of *E. paniculata* in northeastern Brazil. Foraging behavior among floral morphs was measured as the frequency of visits to morphs relative to their frequency in the population (preference) and number of flights between inflorescences of the same versus different morphs (constancy). Pollinator density (number/m<sup>2</sup>/minute) was not correlated with population size, plant density or morph diversity. Pollinator densities varied most among populations of less than 200 plants. Whether pollinators discriminated among the morphs, depended on whether they primarily collected nectar or pollen. In four populations, nectar-feeding bees (*Ancylloscelis* and *Florilegus* spp.) and butterflies showed no consistent preference or constancy among the morphs. In contrast, pollen-collecting bees (*Trigona* sp.) visited a lower proportion of long-styled inflorescences than expected and tended to visit more mid- and short-styled inflorescences in succession, once they were encountered. Pollinator constancy for morphs did not result from differences in inflorescence production or spatial patchiness among the morphs. Although non-random pollinator visitation to morphs in heterostylous populations could potentially affect mating and hence morph frequencies, the observed visitation patterns in this study do not provide evidence that pollinators play a major role in influencing floral morph frequencies.

**Key words:** *Eichhornia paniculata* – Tristyly – Pollination – Northeastern Brazil – Constancy – Behavior

Animal pollinators can be an important cause of variation in reproductive success within zoophilous plant species (Primack and Silander 1975; Motten et al. 1981; Schemske and Horvitz 1984). In contrast to passive vectors such as wind or water, animal pollinators introduce an added complexity because they can respond differentially to floral variation such as color (Waser and Price 1981; Stanton and Preston 1987), scent (Galen 1985), nectar (Thomson and Plowright 1980; Zimmerman 1983) and morphology (Galen 1989; Harder 1990). Moreover, because of variation in environmental (Corbet 1990) and demographic factors (Levin and Kerster 1969; Handel 1983), they can modify their patterns of visitation depending on local ecological conditions. As a result, pollinators can influence the levels of fertility among individuals (Galen 1989; Stanton et al. 1989) as well as the genetic relationships among mates (Ellstrand et al. 1978; Brown and Clegg 1984). Variation in the number and types of pollinators visiting populations not only influences reproductive success but may also be an important selective force in the evolution of the mating system (Campbell and Waser 1987; Harder and Thomson 1989).

*Eichhornia paniculata* (Spreng.) Solms. (Pontederiaceae) is a self-compatible, emergent aquatic of seasonal pools, ditches and low-lying pastures in northeastern Brazil and the Caribbean islands of Cuba and Jamaica. In northeastern Brazil, populations most often contain three floral morphs (long-, mid-, short-styled; hereafter L,M,S morphs); however, some are missing the S morph and occasionally both the L and S morphs. This heterogeneity is associated with the evolution of self-fertilization (Barrett et al. 1989). Tristylous populations are predominantly outcrossing, whereas those that are dimorphic or monomorphic for style length exhibit moderate to high self-fertilization (Barrett and Husband 1990). Variation in selfing rate partly results from genetic modifications to stamen position in the M morph, which favor self-pollination. Observed morph frequencies in *E. paniculata* populations differ from equality, which is predicted by population genetic models for large tristy-

lous populations with no fitness differences among the morphs (Heuch 1979).

The diversity and foraging behavior of pollinators visiting the floral morphs of heterostylous plants have been proposed as important influences on mating patterns and hence morph frequencies in natural populations (Baker 1958; Ornduff 1975; Charlesworth 1979; Beach and Bawa 1980; Weller 1981; Wolfe and Barrett 1987; Muenchow and Grebus 1989). The observed variation in morph frequency and outcrossing rate among populations of *E. paniculata* may therefore result from the patterns of insect visitation within populations and their foraging behavior on the floral morphs. Earlier work on a single population of *E. paniculata* indicated that the absence of specialist, long-tongued pollinators reduced female fertility of the S morph relative to the L and M morphs. This may contribute to the frequent loss of the S morph from populations (Barrett et al. 1989). Additionally, it was suggested that selfing variants may spread in non-trimorphic populations of *E. paniculata* because of unreliable pollinator service owing to the small size of these populations (Barrett 1989).

To evaluate the importance of pollinators to mating-system variation and evolution in *E. paniculata*, we undertook a study of the patterns of insect visitation to populations in northeastern Brazil. Our investigation had two major objectives. First, we wanted to determine levels of pollinator activity in *E. paniculata* populations that differed in size, plant density and floral morph frequency. If unpredictable pollinator service in small populations favors self-pollinating variants (Jain 1976; Lloyd 1980), then small trimorphic populations should have lower and/or more variable pollinator densities. Our second objective was to determine whether pollinators in trimorphic populations preferentially visited the floral morphs, and if such patterns differed among pollinator types (eg. nectar-feeding, long-tongued bees and butterflies versus pollen-collecting, short-tongued bees). Because non-random foraging by pollinators might also result from differences in the spatial distribution of floral morphs within populations, we also investigated the spatial autocorrelation of morphs.

## Methods

We observed insect pollinators in 19 populations of *Eichhornia paniculata* during May–June, 1988 and 1989. These populations were located throughout the range of the species in northeastern Brazil with four, five, three, four and three populations sampled in Ceará, Pernambuco, Alagoas, Sergipe and Bahia states, respectively. In this region, *E. paniculata* primarily receives visits from nectar-feeding, long-tongued bees (*Florilegus* and *Ancyloscelis* spp.) and pollen-collecting bees (*Trigona* sp.). Minor visitors include *Apis mellifera* and various butterflies. Further details on the pollination biology of *E. paniculata* and information on its geographical distribution, reproductive ecology and mating system can be found in Barrett (1985), Barrett et al. (1989), and Barrett and Husband (1990). Observations were made during peak flowering, on sunny days between 0800 and 1130 hrs. Individual flowers bloom for six hours, with most flowers having senesced by 1400 h.

## Survey of pollinator activity

We measured pollinator visitation rates in 16 populations to investigate the relationships between pollinator activity and plant population size, plant density and morph diversity. The number of insects visiting flowers in three to four 1 m<sup>2</sup> quadrats was recorded for three to four 30 s periods each. All values were subsequently converted to a mean number of insects/m<sup>2</sup>/minute for analysis. For each plant population, the number of flowering individuals, their density and the relative frequency of floral morphs were estimated as described by Barrett et al. (1989). Associations between visitation rate and population size, plant density and morph diversity were evaluated using Spearman's rank correlation (Sokal and Rohlf 1981).

To determine whether insects discriminate among the floral morphs of *E. paniculata*, we quantified flower visitation sequences in five trimorphic populations. The populations examined were visited by contrasting insect types. In three populations, insects primarily collected nectar: long-tongued solitary bees dominated the visitors at two populations (B165, B148), whereas butterflies predominated at the other (B151). A fourth population (B94) primarily received visits from pollen-collecting bees, while both pollen- and nectar-collecting bees visited B142, allowing observations on both groups. In each population, we followed insects as they moved through a 7 × 7 m plot and recorded the floral morph and the number of flowers per inflorescence in sequence. This data was used to determine pollinator preference for and constancy on each morph.

## Pollinator preferences

To determine whether insects exhibited a preference among the floral morphs, we compared the frequency of visits to each morph to expected values based on floral morph frequencies within each plot. The significance of deviations from expected visits was analyzed at the level of the inflorescence and flower with goodness-of-fit *G*-tests (Sokal and Rohlf 1981). Since data from each population consisted of several foraging runs, a *G* statistic was calculated for the goodness-of-fit of the pooled frequencies ( $G_p$ ) as well as the heterogeneity among individual runs ( $G_h$ ). Frequencies for some runs were combined for this analysis to ensure that the expected frequencies exceeded five.

## Pollinator constancy

Constancy was measured to determine whether visits to morphs were influenced by the morph of the inflorescence visited previously (Brown and Clegg 1984). The order of pollinator flights to morphs in successively paired visits reflects a pollinator's willingness to change morphs and may influence differential visitation among the morphs and the transfer of pollen between inflorescences of the same morph. Constancy was based on the number of flights between inflorescences of the same versus different morphs. We tested constancy for each floral morph, separately, by using data in which the first of the successive visits was either to L, M or S morphs. In each analysis, flights between the two other morphs were pooled. Observed transitions to same versus different morphs were compared to expected values, calculated from the frequency of visits to each morph in the population with a chi-square goodness-of-fit test with one *DF*. Therefore, any deviation from random in the order of visits to morphs will be independent from results based on pollinator preference, described above.

## Spatial distribution of floral morphs

Non-random distribution of floral morphs within populations may influence foraging patterns of insects, particularly their morph con-

stancy. To compare the spatial dispersion of floral morphs, we measured the correlation between floral morphs of neighboring individuals using spatial autocorrelation analysis for nominal data (Sokal and Oden 1978) in each of five populations. Floral morphs were identified at regularly spaced points along transects (two-dimensional lattices, in three populations). Each sampling point was placed about 0.5 m apart to ensure that inflorescences from different genets were sampled. The length and number of transects depended on the size and shape of each population. All plants connected directly by the transect (rook's moves, Sokal and Oden 1978), were considered neighbors in this analysis. Spatial autocorrelation was estimated as deviations of observed frequencies of like and unlike neighbors from expectations based on a random spatial distribution of morphs using the standard normal deviate. In populations containing all three morphs, six deviations were tested. In addition, the frequency of all unlike joins were combined for a single test of autocorrelation. A significant deficiency of unlike joins would indicate spatial clumping of morphs in a population.

Differences in pollinator constancy for each floral morph could result from unequal production of inflorescences within an individual. To test for differences among morphs in inflorescence production, we counted the number of inflorescences, the total number of flowers/inflorescence and the number of open flowers/inflorescence on randomly selected individuals from three (B142, B148, B165), two (B142, B58) and three (B142, B94, B165) populations, respectively. Differences among morphs were evaluated with an unbalanced, one-way ANOVA (Sokal and Rohlf 1981).

## Results

### Survey of pollinator activity

Between zero and eight insects visited inflorescences of *E. paniculata* within 1 m<sup>2</sup> quadrats per minute (Table 1). Visitation rate was not significantly correlated with plant population size ( $r_s = -0.27$ ,  $P > 0.25$ ,  $N = 16$ ), plant density ( $r_s = 0.23$ ,  $P > 0.25$ ,  $N = 16$ ) or morph diversity

**Table 1.** Pollinator visitation in 16 populations of *Eichhornia paniculata* of contrasting size, density and morph structure. Morph diversity is measured using a normalized index of diversity ( $E$ ) based on Simpson (1949), and ranges from one, in trimorphic populations with equal morph frequencies, to zero, in monomorphic populations. Density is reported on a scale from one (sparsely distributed individuals) to five (dense, continuous population)

Population	Insects/m <sup>2</sup> /min	Size	Density	$E$
B45	0.20	250	3	0.98
B124	0.67	1911	3	0.97
B114	0.94	500	4	*0.68
B94	1.00	200	5	0.83
B167	1.33	4	2	*0.75
B76	1.46	9	1	0.92
B145	1.56	250	4	0.98
B41	1.59	150	4	0.97
B154	1.67	175	3	0.45
B155	2.00	1200	5	0.74
B58	2.33	275	3	0.94
B62	2.40	540	5	0.96
B59	5.67	51	3	*0.74
B120	6.40	100	3	0.67
B151	7.56	200	4	0.78
B93	7.60	30	4	*0.73

\* indicates dimorphic populations with self-pollinating variants

( $r_s = 0.07$ ,  $P > 0.90$ ,  $N = 16$ ). In relatively small populations (e.g.  $\leq 200$ ) insect activity varied considerably, comprising nearly the full range of levels observed. Insect activity did not differ significantly between trimorphic and dimorphic populations (Mann-Whitney  $U$ -test;  $Z = -0.36$ ,  $P > 0.50$ ,  $N_1$  and  $N_2 = 12$  and 4, respectively).

### Pollinator preferences

We observed 116 insect foraging runs that included 1085 inflorescences and 2488 flowers in five populations of *E. paniculata* over two years. Pollen-collecting bees visited the three floral morphs in significantly different proportions than would occur through random foraging (Table 2). In both populations in which pollen-collecting bees were the primary insect visitors, inflorescences of the L morph were undervisited in comparison with their frequency, although in one population (B94) there was significant heterogeneity among foraging runs. The M and S morphs were not visited in a consistent manner in the two populations. In addition, there was a significantly lower number of flowers visited per inflorescence of the L morph in population B94 (Table 3). In both populations, there were highly significant deviations from that expected under random visitation when visits were calculated at the level of the flower (B94, observed flower visits for L, M, and S morphs respectively - 772, 96, 647; expected - 863, 150, 498;  $G = 80.96$ ,  $P < 0.001$ . B142, observed flower visits for L, M, and S morphs respective-

**Table 2.** Frequency of visits to inflorescences of the L, M and S morphs by A) pollen-collecting and B) nectar-feeding insects in five populations of *Eichhornia paniculata*. The  $G$  statistic is presented for data pooled from all foraging runs as well as the heterogeneity among runs within a population

A.								
Morph	Population							
	B142		B94					
	Obs.	Exp.	Obs.	Exp.				
Long	38.0	68.0	166.0	177.8				
Mid	213.0	168.2	18.0	22.2				
Short	107.0	121.7	128.0	111.1				
$N$	312		358					
$G_{het}$	19.1		23.69**					
$G_{pooled}$	28.7**		6.0*					
B.								
Morph	B142		B165		B148		B151	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
Long	26.0	26.0	59.0	43.6	27.0	29.9	45.0	36.2
Mid	59.0	64.4	9.0	13.9	31.0	29.9	54.0	68.9
Short	52.0	46.6	31.0	41.6	7.0	5.2	14.0	7.9
$N$	137		99		65		113	
$G_{het}$	15.7*		10.5*		9.3**		0.1	
$G_{pooled}$	1.1		9.8**		0.9		9.4**	

\* $P < 0.05$ ; \*\* $P < 0.01$

**Table 3.** Mean number of flowers visited per inflorescence by insects on each floral morph of *Eichhornia paniculata*. The five populations examined were visited by either pollen-collecting bees (A) and/or nectar-feeding bees (B). *N* is the number of inflorescences examined in each population. Differences among morphs were statistically significant ( $P < 0.05$ ) in a one-way ANOVA, in only one population (B94). Note that the mean number of flowers/inflorescence did not differ among the morphs in randomly sampled inflorescences (see Results)

Morph	Population					
	B142		B94		B151	
	A.		B.		B.	
Long	4.65	1.67*	2.73	1.58	2.52	1.62
Mid	4.57	2.83*	2.53	1.56	2.13	1.43
Short	5.18	2.75*	2.98	1.72	1.86	1.36
<i>N</i>	312	358	137	100	65	113

ly - 65, 602, 292; expected - 182.2, 450.7, 326.1;  $G = 150.01$ ,  $P < 0.001$ ).

Patterns of foraging by nectar-feeding insects on the floral morphs of *E. paniculata* were less consistent. In two of the four populations, bees showed no preferences among inflorescences of the floral morphs, whereas we detected a preference for the L morph in the remaining two populations (Table 2). The number of flowers visited per inflorescence by nectar-feeding insects did not differ among morphs in the four populations (Table 3).

#### Pollinator constancy

Pollinators exhibited significant morph constancy in three of six populations examined (Table 4). In all cases in which chi-square tests were significant, there was an excess of flights between inflorescences of the same morph and a deficiency of flights between inflorescences of different morphs.

Constancy differed between insect types and among the floral morphs. Butterflies (B151) visited successive inflorescences independently of morph. In contrast, pollen-collecting bees (B94, B142) moved among inflores-

**Table 4.** Constancy in insect visits to floral morphs of *Eichhornia paniculata* in six populations from northeastern Brazil as measured by the frequency of flights from a particular morph to inflorescences of the same versus different morphs. Deviations from expected values, based on the frequency of visits to each morph, are reported as chi-square values. Populations are primarily visited by A) pollen-collecting bees and/or B) nectar-feeding bees and butterflies

Morph	Population					
	B142		B94		B151	
	A.		B.		B.	
Long	0.03	1.70	7.20*	0.02	2.20	0.19
Mid	9.50**	9.70**	1.30	0.13	3.60	2.70
Short	30.20**	3.91*	2.80	0.47	0.47	0.90

\*  $P < 0.05$ ; \*\*  $P < 0.005$

cences of the M morph or among inflorescences of the S morph significantly more often than would be expected with random foraging. This result agrees with data on the frequency of visits among the morphs (Table 2), which indicate that pollen-collecting bees consistently under-visit the L morph. Nectar-feeding bees exhibited marginally significant constancy in one of the three populations examined and only with respect to the L morph.

#### Spatial distribution of floral morphs

Populations of *E. paniculata* showed little spatial structure with respect to floral morphs. Based on the standard normal deviate, four of the five populations exhibited no greater deviation in morph type among neighboring plants than one would expect with random dispersion (Table 5). In one population (B165) there were significantly more joins between inflorescences of the M morph and between inflorescences of the S morph and a deficiency of joins between the L and S morphs and M and S morphs. This indicates a clumping of the M and S morphs but not the L morph. Similar spatial analyses were conducted on an additional eight populations not included in this study (data not shown). In these cases floral morphs exhibited no spatial heterogeneity among neighboring plants.

Analysis of randomly selected individuals indicated no differences among morphs with respect to the number of inflorescences/plant (B142,  $F = 0.49$ ,  $P > 0.50$ ,  $N_L, N_M, N_S = 14, 20, 11$ ; B148,  $F = 0.19$ ,  $P > 0.75$ ,  $N_L, N_M, N_S = 7, 7, 3$ ; B100,  $F = 0.60$ ,  $P > 0.50$ ,  $N_L, N_M, N_S = 33, 33, 15$ ), the total number of flowers/inflorescence (B142,  $F = 0.49$ ,  $P > 0.50$ ,  $N_L, N_M, N_S = 14, 20, 11$ ; B58,  $F = 0.74$ ,  $P > 0.25$ ,  $N_L, N_M, N_S = 15, 15, 16$ ), and the number of open flowers/inflorescence (B142,  $F = 1.49$ ,  $P > 0.10$ ,  $N_L, N_M, N_S = 17, 37, 13$ ; B94,  $F = 1.64$ ,  $P > 0.10$ ,  $N_L, N_M, N_S = 40, 5, 25$ ; B165,  $F = 0.34$ ,  $P > 0.50$ ,  $N_L, N_M, N_S = 81, 24, 78$ ).

**Table 5.** Spatial dispersion of style morphs from five trimorphic populations of *Eichhornia paniculata*. Spatial autocorrelation is reported as the deviation between observed and expected number of joins between like and unlike morphs, transformed to a standard normal deviate. The standard normal deviate is also presented for the total number of joins between unlike morphs, regardless of which pair is involved (Sokal and Oden 1978). Asterisks indicate joins that occur significantly more (+) or less (-) frequently ( $P < 0.05$ ) than a random expectation

Join type	Population				
	B94	B133	B145	B151	B165
L-L	1.35	0.42	-0.85	0.52	1.98
M-M	-0.36	-0.47	0.56	0.11	2.37*
S-S	-0.11	0.83	0.26	-0.37	3.69*
L-M	-0.38	0.24	0.50	-0.39	0.54
L-S	-1.12	-1.06	0.33	-0.14	-2.72*
M-S	1.08	0.33	-0.79	0.43	-2.43*
Total	-0.85	-0.25	0.09	-0.30	-3.57*
Pairs	49	388	76	103	184

## Discussion

The floral morphs of *E. paniculata* do not differ with respect to flower color and size; however, differences in floral morphology associated with the heterostylous syndrome may cause pollinators to visit the three morphs in a non-random manner. Flowers of heterostylous species possess two (distyly) or three (tristyly) distinct organ levels that may differ in accessibility to pollinators, depending on the floral morphology and the type of pollinators involved. Because the perianth tube in *E. paniculata* narrows at the base, long-level organs are most accessible, whereas short-level organs are positioned deep within the floral tube. Differences in pollen presentation among the three stamen levels may therefore influence pollinator visitation, particularly if pollen is the primary food source. Pollen-collecting bees are known to alter their behavior in response to variation in pollen availability (Harder 1990 and references therein). Morph-specific preferences were observed by Wolfe and Barrett (1987) in *Pontederia cordata* and suggested by Ornduff (1975) for *Lythrum junceum* based on an analysis of corbicular pollen loads.

In *E. paniculata*, pollen-collecting bees preferentially visited inflorescences of the M and S morphs, compared to inflorescences of the L morph (Table 2). Bees also tended to visit more flowers on M and S inflorescences than on L inflorescences (Table 3) and showed greater constancy toward inflorescences of M and S morphs (Table 4). These data suggest that variation in pollen presentation among the floral morphs of heterostylous plants influences the foraging behavior of pollinators. To the extent that pollinator preferences influence mating systems, the observed preferences for M and S morphs could have potentially important ecological and evolutionary consequences. Several models of mating-system evolution in heterostylous plants have been based on the assumption that morph-specific differences in pollinator behavior occur (Baker 1958; Charlesworth 1979; Beach and Bawa 1980; Muenchow and Grebus 1989). Few data on pollinator visitation patterns in heterostylous plants have been available, however, to evaluate these ideas (although see Weller 1981).

In contrast, such morph preferences are not expected for nectar-feeding pollinators since sugar concentration and volume does not vary among the morphs of heterostylous species where it has been examined (Weller 1981; Wolfe and Barrett 1987). Insects visiting *E. paniculata* that feed primarily on nectar did not consistently treat the three floral morphs differently with respect to the number of inflorescences visited (Table 2), the number of flowers visited per inflorescence (Table 3) or the number of inflorescences of a morph visited in succession (Table 4). Similarly, in *P. cordata* nectar-feeding *Bombus* spp. visited the floral morphs without preference in two Ontario populations (Price and Barrett 1982; Wolfe and Barrett 1987).

Insects visiting heterostylous populations may exhibit constancy with respect to morphs because of their spatial aggregation, due to clonal growth and near-neighbor foraging (Levin and Kerster 1974). Hence, constancy

may occur passively, rather than through an active search for flowers with a particular morphology (Thomson 1980). The apparent pollinator preferences for floral morphs of tristylous *Oxalis alpina* likely resulted from localized foraging behavior of individual bees and the spatial segregation of morphs due to clonal growth (Weller 1981). Morph constancy was also attributed to the spatial clumping of color morphs in *Ipomoea purpurea* (Brown and Clegg 1984).

In *E. paniculata*, the observed constancy to M and S morphs by pollen-collecting bees did not result from spatial aggregation of morphs since spatial autocorrelation analysis among nearest neighbors indicated negligible patchiness. In the single population exhibiting patchiness (B165), insects did not exhibit constancy on any of the morphs. Lack of patchiness is not unexpected, since *E. paniculata* is primarily annual in northeastern Brazil, with no clonal growth. Furthermore, differences in constancy among the floral morphs probably did not arise because of visitation to multiple inflorescences per plant as inflorescence production did not differ among the morphs. The observed constancy, therefore, probably results from foraging decisions made by pollen-collecting bees, presumably because M and S morphs offer more accessible pollen.

What role does differential pollinator visitation play in determining morph frequencies in *E. paniculata* populations? If pollen-collecting insects preferentially and consistently visit M and S morphs and visitation adequately reflects pollen dispersal in natural populations, the L morph could exhibit reduced fertility, particularly lower male reproductive success. In most populations, however, the S morph, not the L morph, is underrepresented. Furthermore, the only measured reductions in fertility involve the S morph, not the L and M morphs (Barrett et al. 1989). Hence, the preferential foraging behavior of pollen-collecting bees observed in this study probably has not significantly affected the mating system of most tristylous populations of *E. paniculata*.

Assurance of reproduction when mating between individuals becomes difficult is commonly invoked to explain the evolution of self-fertilization in animal-pollinated plants (Jain 1976). Unreliable pollinator service has been suggested as the primary selective force driving the evolution of self-pollination in *Leavenworthia* spp. (Lloyd 1965), *Limnanthes floccosa* (Arroyo 1973), *Lycopersicon pimpinellifolium* (Rick and Fobes 1975), *Gilia achilleifolia* (Schoen 1982) and *Arenaria uniflora* (Wyatt 1986). In *E. paniculata*, self-fertilizing populations tend to be smaller and often less dense than outcrossing populations, a pattern consistent with the reproductive assurance hypothesis (Barrett et al. 1989, Barrett and Husband 1990). If reproductive assurance has been important in the evolution of self-pollination in *E. paniculata*, then pollinator activity should be lower, or perhaps more variable, in small populations. Indeed in Jamaica, where *E. paniculata* populations are highly selfing and usually small in size (Husband and Barrett 1991), pollinator visitation is extremely low and specialized long-tongued pollinators absent (Barrett 1985).

In our study in northeastern Brazil, insect visitation

to *E. paniculata* was uncorrelated with plant population size or plant density. In addition, pollinator activity did not differ significantly between trimorphic populations and those containing selfing variants. However, insect visitation rates were more variable among small populations, a pattern consistent with the reproductive assurance hypothesis. The failure to detect a relationship between pollinator densities and the parameters measured may be associated with the short duration of observations in each population. Since pollinator visits commonly fluctuate daily and seasonally (e.g. Pleasants 1981; Wolfe and Barrett 1988) our extensive (16 populations) rather than intensive sampling may have been too coarse to detect population-level differences in pollinator abundance.

Attempts by other investigators to relate levels of pollinator activity to selection for reproductive assurance have also been largely unsuccessful (e.g. Rick et al. 1978; Wyatt 1986). Collectively, these results highlight the difficulty in testing an hypothesis based on variation in pollinator abundance. Observed pollinator visitation in a population may not reflect the conditions under which self-pollinating variants initially established (Lloyd 1965). During early phases of colonization, when population numbers are low, selfing variants probably have the greatest selective advantage if pollinator service is unpredictable. However, following establishment, populations may increase in size to form sufficiently large floral displays that they attract many insects. Hence the periods during which selection for selfing variants occur may be transient or occur infrequently in a population's history.

*Acknowledgements.* We thank William Cole for field assistance, George Eickwort for insect identification, Lawrence Harder for constructive comments on the manuscript, Josh Kohn for useful discussions, and an operating grant to SCHB from the Natural Sciences and Engineering Research Council of Canada for financial support.

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