Effects of flower number and position on self-fertilization in experimental populations of *Eichhornia paniculata* (Pontederiaceae)

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Summary

1. We examined the effects of daily inflorescence size (three-, six-, nine- and 12-flowered) and the position of flowers within an inflorescence (bottom, middle and top) on the frequency of self-fertilization using genetic markers and experimental manipulation of garden populations of *Eichhornia paniculata*, a self-compatible bee-pollinated plant.

2. Based on the observed tendency for bees to forage upwards on inflorescences and a model of the relation between pollen carry-over and the number of flowers visited per inflorescence, we predicted that the frequency of self-fertilization should increase from bottom to top flowers and with increasing inflorescence sizes.

3. Electrophoretic analysis of open-pollinated progeny arrays supported both of these predictions. The fraction of self-fertilized seeds increased progressively from bottom to top flowers within an inflorescence and there was a significant increase in the frequency of self-fertilization with daily inflorescence size. Inflorescences of all sizes exhibited equivalent increases in the frequency of self-fertilization of flowers from bottom to top positions.

4. The general agreement between our experimental results and model expectations emphasizes the strong influence of pollinator behaviour on mating patterns in self-compatible plants. Such effects have the potential to act as strong selective forces maintaining both anti-selfing mechanisms in mass-flowering species and protandry in species with vertical inflorescences visited by negatively geotactic pollinators.

Key-words: Geitonogamy, inflorescence size, pollen carry-over, pollinator behaviour

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Introduction

Plant reproductive success is affected by two aspects of inflorescence size. The number of flowers open simultaneously (*daily inflorescence size*) can influence pollinator attraction (Carpenter 1976; Augspurger 1980; Thomson 1988; de Jong *et al.* 1992a), the amount of pollen leaving a plant on each pollinator, which should affect the success of pollen dispersal (Lloyd 1979; Bertin 1988; Harder & Thomson 1989; Devlin, Clegg & Ellstrand 1992), and the likelihood of self-pollination (Bowers 1975; Arroyo 1976; Hessing 1988; Schoen & Dubuc 1990). In contrast, the total number of flowers produced by an inflorescence (*total inflorescence size*) is more likely to influence fruit dispersal, by altering the attractiveness of the fruit crop to dispersers (Howe & vande Kerckhove 1979; Davidar & Morton 1986; Denslow 1987), but has little effect on pollination, except through flower dispersion. In mass-flowering species these components of flower production probably do not interact symmetrically to determine reproductive success. In particular, daily inflorescence size is likely to affect the quality and quantity of fertilizations with little influence from total inflorescence size.

Although the effects of daily inflorescence size on the quantity of fertilizations have received considerable attention in mass-flowering species (e.g. Wyatt 1982; de Jong, Klinkhammer & van Staalduinen 1992b; Robertson 1992), its effects on the quality of fertilizations are less well understood. In self-compatible species, differences in the amount of self- and outcross pollen deposited on stigmas can have important functional consequences for the mating system and hence plant fitness (Lloyd 1980; Schoen 1983; Barrett, Kohn & Cruzan 1992; Holsinger 1992). Flowers of animal-pollinated plants are likely to be
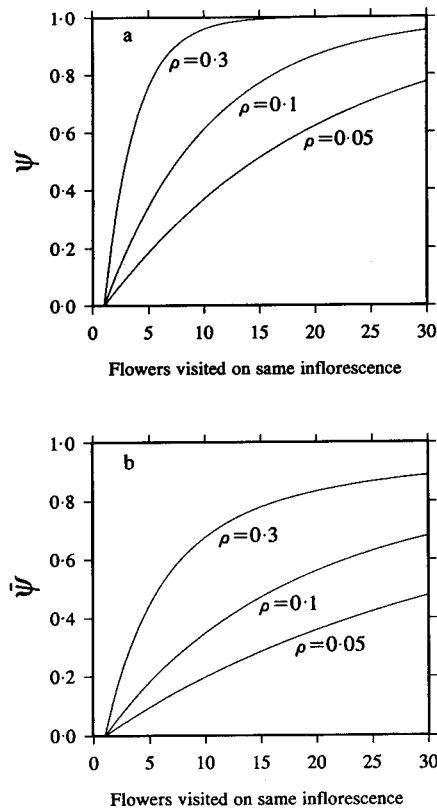
Fig. 1. Expected effects of the number of flowers visited by an individual pollinator within a single inflorescence on (a) the fraction of self-pollen grains deposited on the stigma of each flower (ψ, see equation 2), and (b) the average fraction of self-pollen grains deposited in the inflorescence as a whole ($\bar{\psi}$, see equation 3). The different curves in each panel indicate the role of the proportion of the pollen on a pollinator's body that is deposited on a stigma (ρ) in these processes [data presented in Robertson's (1992) Table 1 generally indicate $\rho < 0.3$]. These examples involve no intrafloral self-pollination (i.e. $I = 0$).

particularly susceptible to differences in self- and out-cross pollen delivery because pollinators often respond to variation in floral display (Andersson 1988; Klinkhammer, de Jong & de Bruyn 1989) and frequently forage directionally within inflorescences (Benham 1969; Pyke 1978). If pollinators visit more flowers on larger plants than small, then rates of between-flower self-fertilization (geitonogamy) will likely be higher for larger plants (Crawford 1984; Geber 1985).

The relation between the number of flowers visited and the proportion of geitonogamously deposited pollen will depend on the pattern of pollen deposition among successively visited flowers (pollen carry-over). For example, consider pollination of a plant that simultaneously displays several nondichogamous flowers. We invoke several simplifying assumptions concerning pollen dispersal for this species. First, the behaviour of pollinators visiting this species does not depend on whether they just arrived from a different inflorescence, or merely moved between flowers

within the inflorescence. Second, of the pollen grains removed from a flower, some fixed number, D, will eventually reach the stigmas of other flowers. This assumption implies the corollary that each flower receives D pollen grains from other flowers during a pollinator visit. Third, during each visit to a flower a fixed fraction, ρ (deposition fraction), of the pollen on the pollinator's body is deposited on the stigma. Finally, each pollinator visit also results in a fixed intensity of intrafloral self-pollination, so that the stigma receives I pollen grains from its own anthers (i.e. facilitated self-pollination; Lloyd & Schoen 1993). Given these assumptions, a pollinator will deposit:

$$d_i = D\rho\,(1-\rho)^{i-1} \qquad \text{eqn 1}$$

of the pollen grains that it removed from flower j on the stigma of the ith flower visited subsequently (see also Galen & Rotenberry 1988; de Jong et al. 1992b). Because of the second assumption, $\rho(1 - \rho)^{i-1}$ has two interpretations: it is equally (1) the fraction of the pollen dispersed from flower j that reached flower i; and (2) the proportion of the pollen received by flower i that originated at flower j. In addition note that $D = \Sigma d_i$.

The total fraction of self-pollen received by the kth flower visited within an inflorescence by a single pollinator (ψ_k) depends on the number of grains transferred from anthers to stigmas within that flower (intraflower self-pollination, I) and the number of grains transferred between flowers within the inflorescence (geitonogamous self-pollination: G_k), so that $\psi_k = (I + G_k)/(I+D)$ Based on equation 1, geitonogamous pollination will deposit:

$$G_k = D\rho \sum_{i=2}^{k} (1-\rho)^{i-2} = D[1-(1-\rho)^{k-1}]$$

self-grains, so that the fraction:

$$\psi_k = \{I+D[1-(1-\rho)^{k-1}]\}/(I+D)$$

$$= 1 - \frac{D(1-\rho)^{k-1}}{I+D} \qquad \text{eqn 2}$$

of all pollen grains received will be self-grains. This relation indicates that the probability of self-pollination increases asymptotically with successive flower visits within an inflorescence because of geitonogamous transfer (Fig. 1a). Furthermore, if the pollinator visits v flowers in the inflorescence, the average fraction of self-pollen grains deposited on a stigma ($\bar{\psi}$) will be:

$$\bar{\psi} = 1/v \sum_{k=1}^{v} \psi_k$$

$$= 1 - \frac{D[1-(1-\rho)^v]}{v\rho(I+D)} \qquad \text{eqn 3}$$

which also increases asymptotically with the number of flowers visited (Fig. 1b). The second term in equation 3 describes the fraction of pollen grains received from other plants. The results of similar analyses by Crawford (1984) and de Jong et al. (1992b) predicted that

the average frequency of self-pollen depends on the proportion of the pollen on a pollinator's body that is deposited on a stigma (p), but not on the number of grains deposited. Equation 3 indicates that such independence with respect to pollen number is expected only in the absence of intrafloral selfing ($I = 0$).

The extent to which daily inflorescence size governs the probability of self-pollination should depend on pollinator behaviour in two ways. First, the overall average probability of self-pollination will increase with daily inflorescence size as indicated by equation 3 only if each pollinator visits all open flowers. The magnitude of deviations from this relation will depend on variation in the number of flowers visited per pollinator and the extent to which the number of flowers visited increases linearly with daily inflorescence size. Second, equation 2 indicates that the probability of self-pollination could vary between flowers within an inflorescence. In particular, pollinators tend to move upwards on vertical inflorescences (Manning 1956; Percival & Morgan 1965; Waddington & Heinrich 1979; Corbet *et al.* 1981; Best & Bierzychudek 1982), so that flowers borne low in the inflorescence should experience less geitonogamous pollen deposition than flowers located higher, at least in species with nondichogamous flowers. Clearly, the occurrence of such consistent, within-inflorescence variation in self-pollination depends on the extent to which different pollinators begin their visits to the inflorescence at the same flower and visit subsequent flowers in the same sequence. These suspected relations of self-pollination to inflorescence size have long been invoked as important selective forces promoting the evolution of various anti-selfing mechanisms, such as self-incompatibility and dioecy in large mass-flowering plants (Darwin 1876; Baker 1959; Maynard Smith 1978) and protandry in species with vertical inflorescences (Darwin 1877; Proctor & Yeo 1973; Richards 1986). However, surprisingly few empirical data have been collected on the effects of flower number and position on plant mating patterns.

We investigated these issues using genetic markers and the experimental manipulation of garden populations of *Eichhornia paniculata* (Spreng.) Solms-Laubach (Pontederiaceae). Our study specifically addressed the following major questions:

1. Does pollinator behaviour vary directly with daily inflorescence size?

2. Does the proportion of self-fertilized seeds within a fruit increase with daily inflorescence size, as implied by equation 3?

3. Do pollinators forage directionally within *E. paniculata* inflorescences?

4. Do flowers borne at the bottom, middle and top of the inflorescence produce different proportions of self-fertilized seeds, as implied by equation 2?

To answer questions 1 and 3 we observed bumblebees (*Bombus* spp.) as they foraged in experimental arrays that differed in flower number. We addressed questions 2 and 4 by collecting open-pollinated capsules from different inflorescence positions on plants in experimental arrays varying in flower number and estimated the proportion of seeds that resulted from self-fertilization using electrophoretic techniques. *Eichhornia paniculata* is well suited to such experiments because: it produces vertical panicle-like inflorescences [see Richards & Barrett (1984) for details of inflorescence morphology] composed of many showy, nondichogamous flowers; individual flowers are short lived (6–8 h) with a new crop of flowers each day; it is self-compatible; self and intramorph (outcross) pollen does not differ in pollen tube growth or siring ability (Cruzan & Barrett 1993); and fertilized seeds rarely abort (Morgan & Barrett 1989; Toppings 1989).

Materials and methods

Experimental arrays were composed of 36 potted plants of the long-styled morph of *E. paniculata* derived from crosses among open-pollinated progeny originating from a natural population (B46) in northeast Brazil. Plants were polymorphic at *AAT-3* and *PGI-2* enabling estimates of the frequency of self-fertilized seeds (s) to be obtained using the multilocus outcrossing rate program of Ritland (1990), where $s = 1 - t$ and t is the mean frequency of outcrossed seeds produced by an array. Standard errors for each estimate of self-fertilization were obtained from 100 bootstraps of the data. Plants in each experimental treatment had a single inflorescence, trimmed to the requisite flower number, and were placed approximately 30 cm apart in a 6×6 grid in a garden at Etobicoke, Ontario. Each array was exposed to pollinators for a single day during August 1991 following protocols described previously by Kohn & Barrett (1992).

Four treatments involving plants with three, six, nine and 12 open flowers per plant were compared to test whether the frequency of self-fertilization increased with daily inflorescence size. On any given day two arrays, each with the same number of flowers per plant and separated by 30 m, were exposed to pollinators for the entire anthesis period of flowers (6–8 h). After they wilted, the flowers open during the experiment were marked with a day-specific paint mark, so that the respective fruits could be identified at maturity. Work to be discussed elsewhere indicates that the two arrays were largely serviced by different individuals of *B. vagans* Smith and *B. fervidus* (Fabricius) and experienced little gene flow between them (<1%), based on studies involving genetic markers. Accordingly, in this study the two arrays were treated independently to examine whether location significantly influenced mating patterns. Each flower number treatment was replicated on 2 separate days during

August 1991 giving a total of 16 arrays (4 inflorescence sizes × 2 locations × 2 days).

For each of the 16 arrays in this experiment we monitored the frequency of bee visits for 10 min during each of the 3 h after the first consistent bee visit of the day. During these observations we recorded the time that each bee entered and left the array and the number of flowers visited per inflorescence by a focal bee. If the focal bee left the array before the end of an observation period, we continued observations with a new focal bee. With these data we calculated the number of bee-minutes experienced by each of the arrays during the total 30 min of observation (e.g. three bees each visiting for 2 min or one bee visiting for 6 min both result in 6 bee-min).

To examine whether bees visited *E. paniculata* inflorescences in a consistent pattern we analysed videotaped records of bees visiting similar 36-plant arrays from a different experiment. The arrays included a mixture of eight- and nine-flowered plants. For each visit to an inflorescence we recorded the positions (numbered from the bottom to the top of the inflorescence) of the first and last flower visited and the number of flights up and down the inflorescence. Based on these data we then calculated medians for each bee, which were used as data in subsequent analyses.

Ten to 12 days after open-pollination three mature capsules were harvested, one each from the bottom, middle and top parts of the inflorescence, of all plants in the arrays. We hypothesized that because bees most commonly forage upwards on inflorescences (see below), bottom flowers would be least likely to experience geitonogamous self-fertilization and top flowers would be most susceptible to geitonogamy (see Fig.1a). Three seeds from all capsules sampled from each array (36 × 3 × 3 = 324 seeds/array) were screened electrophoretically to determine their genotypes at the two marker loci following procedures outlined by Kohn & Barrett (1992).

Seeds from bottom, middle and top capsules were counted from each of five plants in each array to determine whether seed number varied among the experimental treatments. In addition, to determine whether seed set was pollen limited under our experimental conditions, a single flower on five plants in each of four arrays (two six-flowered and two 12-flowered) received supplementary hand cross-pollinations and the seed set of resulting capsules was counted.

Statistical analysis of the data on seed set and the frequency of self-fertilization (s) reflected the repeated-measures design of the experiment which included three between-array factors (flower number, array location and date nested within flower number) and one within-array factor (flower position). Date and its interaction with location were treated as random effects. We accounted for the sampling error associated with each estimate of s by weighting the impact of each estimate in the ANOVA by the inverse of its

squared standard error (see Neter, Wasserman & Kutner 1990). Our expectation of an increased frequency of self-fertilization with increasing daily inflorescence size was tested with an a priori linear contrast (Kirk 1982). Although equation 3 predicts a decelerating relation between the frequency of self-fertilization and daily inflorescence size, Fig. 1b illustrates that this relation is essentially linear over the range of inflorescence sizes examined in this study.

Results

BEE BEHAVIOUR

Daily inflorescence size did not significantly affect the intensity of bee visits to flowers in an array. Based on a two-factor ANOVA, the number of bees entering an array during 30 min of observation did not differ among flower number treatments (Table 1) or between array locations ($P > 0.05$ for both main effects and their interaction). The number of bee-minutes experienced by an array increased significantly with daily inflorescence size (Table 1, $F_{3,8} = 8.54$, $P < 0.01$); however, the number of bee-min per flower did not vary significantly with flower number ($F_{3,8} = 2.23$, $P > 0.1$). These results indicate that bees visited arrays with larger inflorescences longer than those with small inflorescences solely because of differences in flower availability. In particular, the median number of flowers visited per inflorescence generally increased with the number of available flowers, although this trend was less evident among larger inflorescences (Table 1, $F_{3,41} = 3.97$, $P < 0.025$). Neither array location nor its interaction with flower number significantly affected the number of bee-minutes per array or per flower, or the median number of flowers visited per inflorescence ($P > 0.1$ in all cases).

In general, bumble-bees visited flowers on inflorescences of *E. paniculata* from the bottom to the top. The median starting position of nine of the 10 bees (seven *B. fervidus*, three *B. vagans*) for which we videotaped visits to at least five inflorescences involved a flower in the lower third of the inflorescence (flower 3 or lower in eight- or nine-flowered inflorescences, the median starting position for the tenth bee was 3.5). All bees left inflorescences from the upper third of the inflorescence (median leaving position, 7 or higher for all bees). Bees usually visited five (SD = 0.9) of the eight or nine flowers in the inflorescence (mean of median flower visits for each bee) with 3 (SD = 0.7) upward flights and 1 (SD = 0.3) downward flight between flowers.

FREQUENCY OF SELF-FERTILIZATION

Open-pollinated flowers in experimental arrays set significantly fewer seeds per capsule than hand-pollinated flowers, indicating pollen limitation (open-pollinated, mean = 49.0 seeds per capsule, range 47.3–50.7, $n = 248$

Table 1. Aspects of bumble-bee foraging behaviour in relation to daily inflorescence size. The mean and lower and upper standard errors of entries to an experimental array during 30 min of observation and bee-minutes per 10-min observation period are based on arrays in two locations sampled on each of 2 days (i.e. $n = 4$). These statistics are based on square root and log-transformed data, respectively, hence the asymmetric standard errors. The mean ± SE median number of flowers visited per inflorescence is based on the number of bees indicated in parentheses

No. of flowers per inflorescence	No. of bees entering array	Bee-minutes per 10-min observation period	Median no. of flowers visited per inflorescence
3	6·9[a]	5·8[a]	2·0±0·16[a]
	6·13–7·77	5·25–6·45	(13)
6	7·1[a]	6·1[a]	2·5±0·31[a,b]
	5·83–8·43	4·55–8·19	(11)
9	12·9[a]	14·0[b]	3·3±0·33[b]
	10·65–15·41	12·76–15·26	(16)
12	10·7[a]	15·9[b]	3·5±0·29[b]
	8·81–12·81	14·25–17·71	(9)

Superscript letters following each mean indicate the results of Tukey–Kramer multiple comparisons of the different inflorescence sizes: means with different letters differ significantly ($P<0.05$).
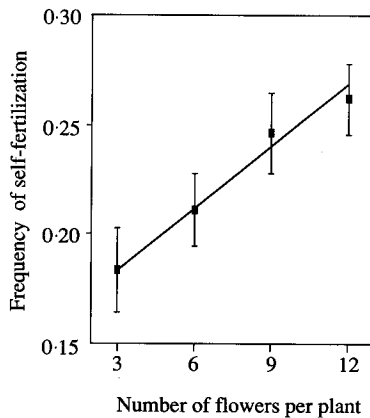

Fig. 2. The effect of daily inflorescence size on the mean frequency of self-fertilization (± SE) in experimental populations of *Eichhornia paniculata* ($n = 4$ replicates per treatment).

capsules; hand-pollinated, mean = 90·3 seeds per capsule, range 86·8–93·9, $n = 20$ capsules, based on square-root transformed data). There was no significant effect of flower position within an inflorescence ($F_{2,64} = 0.005$, $P > 0.99$), daily inflorescence size ($F_{3,4} = 0.49$, $P > 0.5$), array location ($F_{1,4} = 0.16$, $P > 0.5$), date ($F_{4,4} = 4.21$, $P > 0.05$) or their interactions ($P > 0.1$ in all cases) on the number of seeds set per capsule by open-pollinated flowers.

The frequency of self-fertilization increased gradually with daily inflorescence size (Fig. 2). The overall test of the relationship between flower number and the frequency of self-fertilization revealed no significant effect ($F_{3,4} = 3.86$, $P > 0.1$), probably because of limited power resulting from few replicates per treatment (power ≈ 35%). In contrast, the more powerful test for a linear trend revealed that self-fertilization increased consistently with increasing daily inflorescence size (Fig. 2, $F_{1,4} = 11.44$, $P < 0.05$). A

weighted regression based on the means for each flower number treatment ($s = 0.16 + 0.01F$; where F is daily inflorescence size) indicates that each additional flower increased the proportion of self-fertilized seeds by about 1%. Futhermore, based on this regression with $F = 1$, approximately 17% of the seeds matured by a flower resulted from intrafloral self-fertilization.

Flower position affected the frequency of self-fertilization more than daily inflorescence size as the fraction of self-fertilized seeds varied greatly among flowers at different positions within inflorescences (Fig. 3, $F_{2,8} = 44.74$, $P < 0.001$). Bottom flowers were less highly selfed than either middle or top flowers and middle flowers were less highly selfed than top flowers. Because most pollinators moved upwards within an inflorescence, the fraction of self-fertilized
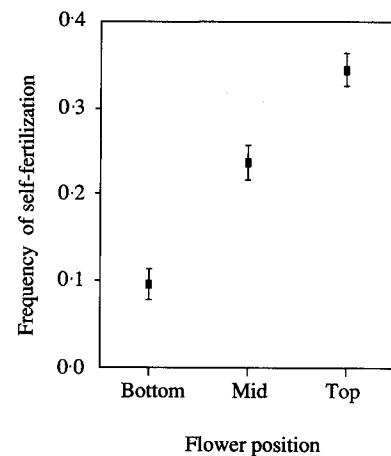

Fig. 3. The effect of flower position within an inflorescence on the mean frequency of self-fertilization (± SE) for *Eichhornia paniculata* flowers borne at the bottom, middle and top of inflorescences. The data are pooled across the four inflorescence size classes.

seeds for bottom flowers (10%) should largely reflect intrafloral self-fertilization. Equation 2 predicts that self-fertilization of top flowers on large inflorescences should be higher than that of top flowers on small inflorescences if the number of flowers visited by each pollinator increased linearly with flower number. However, in our experiments inflorescences of all sizes exhibited equivalent increases in amounts of self-fertilization up the inflorescence, as flower position and flower number per plant did not interact significantly ($F_{6,8} = 1.16$, $P > 0.25$). That top flowers on three-flowered inflorescences experienced a similar frequency of self-fertilization as top flowers on 12-flowered inflorescences probably reflects the limited, nonlinear increase in the number of flowers visited by each pollinator with inflorescence size (Table 1). Array location, sampling date and their interaction with each other and with other effects did not significantly affect the frequency of self-fertilized seeds ($P > 0.15$ in all cases).

Discussion

INFLUENCES ON THE LIKELIHOOD OF SELF-FERTILIZATION

In our experiments involving *E. paniculata*, daily inflorescence size and flower position affected the frequency of self-fertilization as expected from the relation of pollen carry-over to the number of flowers visited within an inflorescence. As predicted from equation 2 (also see Fig. 1a) and the tendency of bees to forage upwards on inflorescences, flowers low on inflorescences produced a smaller fraction of self-fertilized seeds than flowers higher up the inflorescence (Fig. 3). Furthermore, the likelihood of self-fertilization increased with daily inflorescence size, as implied by equation 3 (also see Fig. 1b). Finally, flower position affected the frequency of self-fertilization more strongly than did flower number. Comparison of the rates of increase of corresponding curves in Fig. 1a and b indicate that this greater influence of flower position is also consistent with the role of pollen carry-over in geitonogamous self-pollination. Position-dependent effects on mating patterns are not restricted to animal-pollinated plants; in wind-pollinated conifers, cones lower in the crown experience more self-fertilization than those higher up (Franklin 1971; Squillance & Goddard 1982). This pattern presumably results from the greater likelihood of air-borne pollen being carried downwards than the reverse.

The qualitative agreement of our results with expectations based on equations 2 and 3 emphasizes the dominant effect on self-fertilization of general features of pollinator behaviour, especially the tendency for bees to visit more flowers on large inflorescences and to move upwards within inflorescences. This consistency between results and expectations

occurred even though: (1) bees generally did not visit every flower; (2) the number of flowers visited per inflorescence varied between bees and between inflorescences for the same bee; (3) the number of flowers visited per inflorescence did not increase linearly with inflorescence size; (4) bees differed in their starting positions; and (5) bees did not always move upwards to the next flower in the inflorescence. Furthermore, for bee-pollinated plants the actual pattern of deposition of pollen originating from a specific flower differs in two ways from the pollen carry-over model depicted by equation 1 (also see Fig. 1a). First, even though deposition of pollen from a particular donor generally declines with each subsequent visit to recipient flowers, deposition varies considerably around this trend (reviewed by Robertson 1992). Second, results by Thomson (1986) on pollen carry-over suggest that the fraction of pollen originating from a specific flower that is deposited on the stigma of a particular recipient flower generally declines with successive visits to recipient flowers (i.e. ρ is not constant). Third, each bee visit probably did not deposit a fixed number of pollen grains on a stigma (i.e. D is not constant). The apparent resilience in patterns of self-fertilization, in the face of variable bee behaviour and likely variation in pollen carry-over, implies that flower position and daily inflorescence size should strongly influence the likelihood of self-pollination in plants with vertical inflorescences visited by negatively geotactic pollinators.

Daily inflorescence size differed fourfold in our experiments; however, this represents only a small fraction of the variation found within populations of many self-compatible species (including *E. paniculata* —Morgan & Barrett 1989), particularly those that exhibit mass flowering. Unless such species possess floral mechanisms that reduce the likelihood of geitonogamous pollinations, individuals with large floral displays are likely to experience considerably higher levels of self-fertilization than were observed in our study. In the only other experimental study that we are aware of that has measured flower number per plant and the likelihood of self-fertilization, Crawford (1984) found a significant positive relationship between the two parameters in the bee-pollinated perennial, *Malva moschata*. In contrast to our study, he found a much greater range of selfing rates estimated at the individual plant level ($s = 0$ to approx. 0.80). This variation was associated with differences in flower number ranging from approximately five to 25 per plant.

Our two estimates of intrafloral self-fertilization (10 and 17% of all seeds) imply that the absence of dichogamy in *E. paniculata* flowers enhances the likelihood of selfing. These estimates are probably more reasonably regarded as maxima, rather than means, as some self-pollination could have resulted from bees that revisited flowers low on an inflorescence after visiting a few flowers on a neighbouring inflorescence. With this caution in mind, comparison

of these estimates of intrafloral self-fertilization with the overall selfing frequencies for flowers at different positions (Fig. 3) suggests that geitonogamous pollen transfer equalled intrafloral transfer only for flowers at the top of the inflorescence.

The low average and limited range in frequencies of self-fertilization (range of $s = 0.12–0.35$, $n = 16$ experimental arrays) in our experiments imply that bumble-bees deposited less intrafloral and geitonogamous pollen on stigmas than outcrossed pollen. The likelihood of self-fertilization was probably little influenced by either post-pollination or post-zygotic mechanisms because seed production was pollen limited, self-pollen is not at a competitive disadvantage in fertilization relative to intramorph (outcross) pollen and few seeds abort in this species (see above). Therefore, it seems more likely that the exserted stigma and large stigma–anther separation of approach herkogamous flowers in the long-styled morph of *E. paniculata* served to reduce self-pollen deposition by pollinators.

IMPLICATIONS FOR MATING-SYSTEM STUDIES

Quantitative estimates of the frequency of selfed and outcrossed seeds are now commonly reported in the plant reproductive ecology literature (reviewed in Schemske & Lande 1985; Brown, Burdon & Jarosz 1989; Barrett & Eckert 1990). The availability of isozyme markers provides opportunities to investigate the ecological and genetic basis of mating patterns at various scales including the population, individual and flower levels. However, most studies to date have investigated variation at the population level, by bulk samples of open-pollinated families from natural or experimental populations. These studies usually report point estimates of the mean fraction of outcrossed seeds. Our results suggest that in self-compatible, animal-pollinated species such estimates likely subsume considerable heterogeneity at the individual and flower levels. Such variability has important implications for the collection of seed families and suggests that more attention should be paid to the range of plant sizes and flower positions from which progeny are sampled. For example, in populations with considerable variation in plant size, more accurate estimates of the frequency of self-fertilization will be obtained by sampling a constant proportion of seeds per plant rather than a fixed number (Crawford 1984). Furthermore, models of mating-system estimation that account for variation at the plant (Ritland & Ganders 1985) and flower (Schoen & Brown 1992) levels are likely to be more appropriate for self-compatible species that exhibit mixed-mating systems.

Despite considerable variation in daily weather conditions, levels of pollinator activity, and the potential for local environmental effects associated with the different array locations, neither day nor location significantly affected among-array variation in the frequency of self-fertilization. The reduced magnitude of these 'environmental effects' enabled us to detect a small but significant influence of daily inflorescence size on the frequency of self-fertilization. Our ability to detect this signal on a parameter like s, which in natural populations is notoriously subject to measurement error, is undoubtedly due to the controlled conditions of our experiments. All plants within an array were evenly spaced, of similar height and had an equal number of flowers per plant. In addition, alleles at the marker loci were at similar frequencies in each array and the same number of progeny, equally divided among each of three flowers, were sampled from all plants. Attempts to demonstrate an association between flower number and mating patterns within natural populations using correlative approaches (e.g. Wolfe & Shore 1992) are likely to be a good deal more difficult owing to the presence of many uncontrolled variables and because of the large samples required to estimate mating-system parameters at the individual level (see Morgan & Barrett 1990).

ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS

Geitonogamous self-pollination has at least two effects on the relative advantages of a particular daily inflorescence size. First, daily inflorescence size could influence the opportunities for selective abortion of fruits based on the proportion of self-fertilized seeds (Stephenson 1981; Lee 1988; Becerra & Lloyd 1992). In general, the opportunities for selective abortion will depend on the variation between fruits in seed paternity. Given the role of daily inflorescence size in determining both the proportion of geitonogamous self-pollen deposited on flower k (ψ_k, see equation 2) and the average proportion of self-pollen deposited on all v flowers visited by a single pollinator ($\bar{\psi}$, see equation 3), the between-flower variance in the fraction of self-pollen is:

$$\sigma_\psi^2 = 1/v \sum_{k=1}^{v} (\psi_k - \bar{\psi})^2$$

$$= \frac{D^2}{v^3 \rho^2 (I+D)^2} \sum_{k=1}^{v} [1-(1-\rho)^v - v\rho(1-\rho)^{k-1}]^2 \quad \text{eqn 4}$$

This variance, or the corresponding standard deviation (σ_ψ), varies in a complicated fashion with the number of flowers visited and the proportion of the pollen pool on a pollinator's body deposited on each flower (deposition fraction, ρ). When pollen carry-over is extensive (ρ small), the opportunity for selective abortion, as determined by σ_ψ, increases with the number of flowers visited per pollinator (Fig. 4). However, when pollen carry-over is more restricted ($\rho > 0.1$), the opportunity for selective abortion initially increases rapidly with the number of flowers visited, but then declines gradually as the number of flower visits increases. In general, the number of
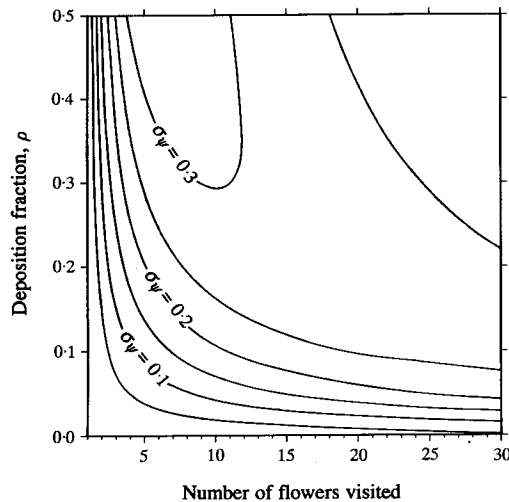
Fig. 4. The expected relation of the standard deviation in the fraction of self-pollen grains (σ_ψ, see equation 4) deposited on stigmas to the number of flowers visited by an individual pollinator within a single inflorescence (v) and the proportion of the pollen on a pollinator's body that is deposited on a stigma (ρ). The isopleths indicate combinations of v and ρ that produce equal σ_ψ. The results assume no intrafloral self-pollination (i.e. $I = 0$).

flower visits that maximizes variation in the proportion of geitonogamous self-pollen varies inversely with the deposition fraction. Because the number of flowers visited generally increases with the number of open flowers, the opportunity for selective abortion should vary in a similar manner with daily inflorescence size.

Second, the relation between the probability of geitonogamous pollination and daily inflorescence size could affect an individual's success as a male parent. If the species involved is self-incompatible, or self-pollen is less successful at siring seeds than outcross pollen, then deposition of self-pollen would represent a loss of paternal opportunities (pollen discounting—Holsinger, Feldman & Christiansen 1984; Ritland 1991). Because the likelihood of geitonogamous pollen deposition increases with the number of flowers visited per pollinator, the negative effects of pollen discounting should promote simultaneous exposure of few flowers to pollinators and extension of the flowering period (also see de Jong *et al.* 1992b). Daily exposure of relatively few flowers to pollinators and prolonged flowering by the entire inflorescence is also an expected outcome of selection that enhances total pollen dispersal when the proportion of successfully dispersed pollen grains varies inversely with the number of pollen grains removed by each pollinator (diminishing returns—Lloyd 1979; Harder & Thomson 1989). Hence the selective influence of pollen discounting should complement selection to ameliorate the effects of diminishing returns through pollen dispersal. However, large floral displays often attract more pollinators than small displays in competitive situations (Augspurger 1980; Cruzan, Neal &

Willson 1988; Klinkhammer & de Jong 1990 and references therein), so that the evolution of daily inflorescence size should reflect compromises between increased pollinator attraction, on the one hand, and increased pollen loss through discounting and other features of pollen dispersal, on the other.

Current studies in plant reproductive ecology often emphasize either the ecological influences on pollen dispersal and seed production or the genetic causes and consequences of various patterns of mating and gene flow. These contrasting approaches have frequently led to a separation between studies of pollination biology from those concerned with plant-mating systems. This dichotomy was not evident in early studies of plant reproduction (e.g. Darwin 1876, 1877) and our theoretical and empirical results indicate that such a distinction is largely artificial. Rather, studies that simultaneously examine the ecological determinants of pollination success and their genetic and evolutionary implications are likely to be most effective at elucidating the selective mechanisms responsible for the diversity of floral strategies found in angiosperm species.

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