

Pollen Dispersal and Mating Patterns in Animal-Pollinated Plants

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Immobility complicates mating by angiosperms because the transfer of male gametes between individuals requires pollen vectors. Although abiotic and biotic vectors can transport pollen considerable distances (Bateman, 1947a; Squillace, 1967; Kohn and Casper, 1992; Godt and Hamrick, 1993), the resulting pattern of pollen dispersal does not intrinsically maximize the number and quality of matings. Consequently, floral evolution generally involves two classes of adaptations that promote mating success. The morphological traits that characterize floral design and display modify the actions of pollen vectors so as to enhance fertility (see below). In contrast, physiological traits mitigate unsatisfactory pollen dispersal by rejecting unsuitable male gametophytes (Jones, 1928; de Nettancourt, 1977; Marshall and Ellstrand, 1986; Seavey and Bawa, 1986; Barrett, 1988; Snow and Spira, 1991; Walsh and Charlesworth, 1992) or zygotes (Stephenson, 1981; Casper, 1988; Becerra and Lloyd, 1992; Montalvo, 1992). As a result of postpollination processes, the realized mating pattern does not simply mirror the pattern of pollination (e.g., Campbell, 1991; also see Waser and Price, 1993). However, these processes can only filter the incipient mating pattern established during pollination, so that pollination fundamentally determines the maximum frequency and diversity of mating opportunities. Consequently, the role of pollination in governing the scope for mating inextricably links the evolution of pollination and mating systems.

Most plant species employ animals as pollen vectors because the behavioral flexibility of animals disposes them to manipulation by plant characteristics. Animal pollination produces a characteristic mating pattern that generally reflects both the tendency of pollinators to move among neighboring flowers and plants

(reviewed by Levin and Kerster, 1974; Handel, 1983; Richards, 1986) and the residence of some of a flower's pollen on a pollinator during succeeding visits to other flowers (pollen carryover; reviewed by Robertson, 1992). These features typically result in local cross-pollination (Schaal, 1980; Handel, 1982; Thomson and Thomson, 1989; Devlin and Ellstrand, 1990; Meagher, 1991; Campbell, 1991) and self-pollination, due partly to pollen transport among flowers on the same plant (geitonogamous pollination; reviewed by de Jong et al., 1993; see also Chapter 7). For self-compatible species, such a pollination pattern often results in a mixed-mating system (reviewed by Schemske and Lande, 1985; Barrett and Eckert, 1990), including some biparental inbreeding (Ritland and Ganders, 1985; Waller and Knight, 1989). In addition, stigmas of animal-pollinated plants often receive many pollen grains from each of several different donors, so that maternal plants produce more full sibs (correlated mating) than would be expected from random mating (Schoen and Clegg, 1984; Schoen, 1985; Ritland, 1989). Hence, animal pollination delivers a complex mixture of pollen from many sources, which in turn facilitates the implementation of diverse mating systems.

Plants govern their mating opportunities through the effects of floral design and display on pollinator attraction and pollen dispersal. Enhanced attraction obviously favors both male and female function when pollination is insufficient to fertilize as many ovules as plants can develop into seeds (see also Chapter 12). Even if pollen receipt does not limit seed production, increased pollinator attraction can benefit male function if increased pollen removal decreases the proportion of removed grains that fertilize ovules and floral mechanisms restrict removal by individual pollinators (Harder and Thomson, 1989; Harder and Wilson, 1994). Attraction of many pollinators and restricted pollen removal also increase potential mate diversity because, with many pollinators following dissimilar foraging paths, an individual plant imports pollen from and exports pollen to a larger sample of the population. Floral characteristics influence pollinator attraction by providing resources of value to animals (e.g., nectar, pollen, floral oils, etc.; reviewed by Simpson and Neff, 1983) and by signaling the location of these resources with visual and/or olfactory displays (e.g., showy perianths, nectar guides, floral odors, many-flowered inflorescences; see Waser and Price, 1983a; Galen, 1985; Galen and Newport, 1987; Thomson, 1988; Stanton et al., 1989).

Once a pollinator has been attracted, floral traits affect the success of pollen dispersal by determining the amount of pollen exchanged between each flower's sexual organs and the pollinator. Flowers influence pollen removal by each pollinator through the schedule of pollen presentation and dispensing (Harder and Thomson, 1989; Armstrong, 1992; Harder and Barclay, 1994; LeBuhn and Anderson, 1994; Harder and Wilson, 1994) and by controlling reward availability, which affects the duration of pollinator visits (e.g., Harder, 1990a; Young and Stanton, 1990). The site of contact between the pollinator and the flower's

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pollen-presenting structures also affects pollen removal (Harder, 1990a; Murcia, 1990; Wilson and Thomson, 1991; Harder and Barrett, 1993) and additionally determines the susceptibility of transported pollen to pollinator grooming or burial by pollen from subsequently visited plants. Recipient flowers influence pollen deposition through the duration and site of contact between the stigma and pollinator, which depend on the flower's control of pollinator position and on stigma size and position (Galen and Plowright, 1985; Murcia, 1990; Johnston, 1991). Thus, although flowers interact with pollinators only briefly, the diverse influences of flower characters enable flowers to manipulate patterns of pollen dispersal.

Even though the pattern of pollen dispersal determined by the interaction between pollinator and floral characteristics circumscribes the opportunities for mating between plants, the relation between pollination and mating is seldom the focus of ecological and evolutionary studies of plant reproduction (for exceptions, see Levin and Kerster, 1974; Waser and Price, 1983b; Brown and Clegg, 1984; Richards, 1986; Abbott and Irwin, 1988; Campbell, 1991; Stanton et al., 1991; Holsinger, 1992; Lloyd and Schoen, 1992; Waser, 1993; Kohn and Barrett, 1994). On one hand, pollination biologists emphasize floral characters and their influence on pollinator visitation, pollen export and seed production by individual plants, but they typically pay little attention to who has mated with whom. On the other hand, mating-system biologists generally focus on mating patterns at the population level, especially the relative frequency of selfing vs. outcrossing, with less regard for the processes producing those patterns. Consequently, modern studies of the ecology and evolution of plant reproduction often tend to disarticulate reproductive processes from their outcomes.

The relative isolation of studies of pollination and mating systems reflects the separate development of ecological and genetic perspectives that has characterized the history of evolutionary biology in general. Pollination biology developed from the natural-history tradition established by Sprengel, Darwin, F. and H. Müller, Delpino, Knuth, and others during the nineteenth century (reviewed by Baker, 1983) and maintains a primarily empirical approach to analyzing ecological aspects of plant-pollinator interactions and their consequences for reproduction under natural conditions. In contrast, mating-system biology developed during the middle of the twentieth century as a component of population genetics, and uses laboratory-intensive techniques to examine formal theoretical problems. Hence, pollination and mating-system biology arose from different, but complementary, perspectives on plant reproduction. Nevertheless, the isolation of pollination and mating-system biology hinders comprehensive understanding of the function and evolution of floral characters that influence fertility.

Our objective in this chapter is to illustrate how analysis of the relation between pollination and mating can clarify the functional significance of floral design and display. As our primary example, we consider the effects of the number of flowers that a plant exposes simultaneously (daily inflorescence size) on pollinator

attraction, pollen dispersal, and the resulting mating pattern, especially the relative frequency of selfing and outcrossing. We begin with a brief overview of the influences of daily inflorescence size on pollination and mating. We then temporarily step back from the inflorescence perspective and examine pollen dispersal among individual flowers to illustrate theoretically and empirically how characteristics of flowers and their pollinators determine the dispersion of donor pollen among recipient flowers. Given this background in pollen dispersal, we then return to the question of how the arrangement of flowers into inflorescences influences mating patterns. In particular, we predict the influence of inflorescence size on various mating parameters, based on a simplified model of pollen carryover, and we then test many of these predictions with an empirical study of the role of daily inflorescence size on mating in experimental arrays of *Eichhornia paniculata* (Pontederiaceae). To illustrate some evolutionary consequences of the interaction between pollination and mating, we also present a cost-benefit analysis of daily inflorescence size. Based on our analysis of the dependence of mating on pollination, we propose novel interpretations of several aspects of floral design and display, including sterile flowers, dichogamy, and heterostyly.

General Influences of Daily Inflorescence Size on Pollination and Mating

Most plants display several flowers at one time, so that their mating patterns depend on the collective contributions of individual flowers to pollination and seed production. Such aggregated flowering complicates the influences on plant fertility because flowers do not function in isolation from other flowers within an inflorescence. These complications arise primarily from the relation of pollinator attraction and geitonogamous pollen transfer to daily inflorescence size.

Large inflorescences often attract more pollinators than small inflorescences (Schaffer and Schaffer, 1979; Augspurger, 1980; Paton and Ford, 1983; Schmitt, 1983; Bell, 1985; Geber, 1985; Andersson, 1988; Cruzan et al., 1988; Thomson, 1988; Klinkhamer et al., 1989; Klinkhamer and de Jong, 1990; Eckhart, 1991; see also Chapter 7) because the proximity of many flowers reduces pollinator flight costs. However, individual flowers receive more visits only when each pollinator visits a fixed or increasing proportion of the available flowers. Many pollinators do not increase the number of flowers visited per inflorescence in proportion to increases in daily inflorescence size, so that the number of pollinator visits per flower often declines with increasing inflorescence size (e.g., Schmitt, 1983; Geber, 1985; Andersson, 1988; Klinkhamer et al., 1989; Robertson, 1992). Consequently, if daily inflorescence size does not affect pollen removal and deposition during each flower visit, then increased flower display could reduce mating frequency.

Regardless of whether the proportion of visited flowers changes, increases in the number of flowers visited per pollinator with increasing daily inflorescence size generally increase geitonogamous self-pollination (reviewed by de Jong et al., 1993; also see Barrett et al., 1994; Hodges, 1995). The mating consequences of geitonogamy depend on whether such self-pollination directly reduces the amount of pollen reaching other plants (pollen discounting; Holsinger et al., 1984), the relative susceptibility of self pollen to self-incompatibility (reviewed by de Nettancourt, 1977; Barrett, 1988), and the severity of inbreeding depression suffered by selfed offspring (reviewed by Charlesworth and Charlesworth, 1987).

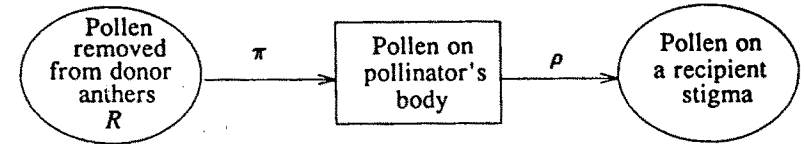
Pollen-Dispersal Between Individual Flowers

The roles of floral design and display in mating are most readily appreciated by first identifying floral influences on pollen dispersal. Unfortunately, although pollen carryover determines the incipient mating pattern, most studies of this process have focused on the extent of carryover, with less emphasis on its fundamental influences (although see Lertzman and Gass, 1983; Waser and Price, 1984; Galen and Plowright, 1985; Thomson, 1986; Feinsinger and Busby, 1987). Despite the absence of explicit functional studies of pollen dispersal, published descriptions of the likely underlying mechanisms can be formalized mathematically to examine how specific interactions between flowers and their pollinators might affect the pattern of pollen dispersal. We therefore briefly describe two models of dispersal. The first model proposes that the pollen on a pollinator constitutes a single, homogeneous pool (single-compartment model; see Fig. 6.1a). This model captures general features of pollen dispersal and is the most commonly used theoretical description of pollen carryover (e.g., Bateman, 1947b; Plowright and Hartling, 1981; Crawford, 1984; Geber, 1985; de Jong et al., 1992; Robertson, 1992; Barrett et al., 1994). However, observed pollen carryover is often more extensive than predicted by the single-compartment model (Thomson, 1986; Morris et al., 1994; Harder and Wilson, unpublished), implying that pollen dispersal is not merely a simple decay process. Therefore, the second model that we consider describes one mechanism that complicates pollen dispersal, namely, frequent grooming by pollinators (two-compartment model; Fig. 6.1b). To evaluate these alternative perspectives of pollen dispersal, we also compare the patterns of dispersal predicted by the one- and two-compartment models with observed dispersal of *Pontederia cordata* L. (Pontederiaceae) pollen by bumble bees. This example also illustrates that floral morphology strongly affects patterns of pollen dispersal.

Single-Compartment Model

The simplest representation of pollen dispersal considers pollen on the pollinator's body as a single, completely mixed pool (Fig. 6.1a). While visiting a flower,

a Single-compartment model



b Two-compartment model for grooming pollinators

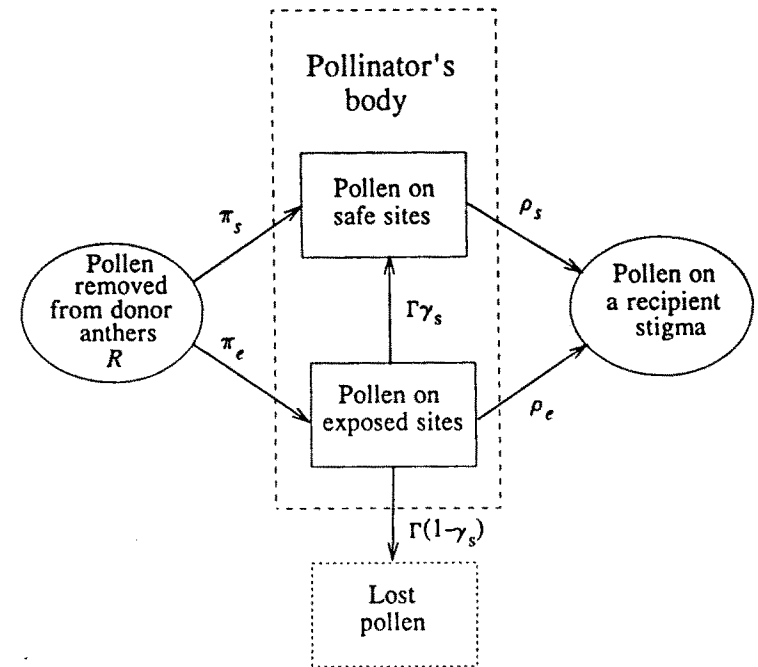


Figure 6.1. Two models of pollen dispersal; (a) Each pollinator carries a single, mixed pool of pollen; or (b) each pollinator carries pollen in a pool exposed to grooming and a pool safe from grooming. Greek symbols indicate transfer probabilities between states during each flight between flowers.

the pollinator removes R pollen grains, of which a fraction π remains on the pollinator as it moves to the next flower. Simultaneously, the flower's stigma removes a fixed proportion ρ (deposition fraction) of the pollen already on the pollinator's body, so that the fraction $1 - \rho$ is carried over for deposition on a subsequently visited recipient. These dynamics apply to all pollen carried by a pollinator; however, let us focus on the πR grains that leave a specific donor flower. The first recipient flower visited receives $d_1 = \rho\pi R$ of these grains, thereby reducing the donor pollen pool to $\pi R(1 - \rho)$ grains. The second recipient flower receives $d_2 = \rho\pi R(1 - \rho)$ donor grains and further depletes the donor pollen pool to $\pi R(1 - \rho)^2$ grains, so that the third recipient receives $d_3 = \rho\pi R(1 - \rho)^2$ donor grains, etc. In general, recipient k receives

$$d_k = \rho\pi R(1 - \rho)^{k-1} \quad (1)$$

pollen grains from the donor of interest.

The pollen from a specific donor flower on the pollinator's body is not replenished, so that repeated deposition of pollen on stigmas causes a continual decline in both the donor pollen pool and the number of donor grains deposited on successive recipient flowers. Based on Equation (1), deposition of donor pollen on the k th recipient flower (d_k) increases linearly with the number of donor grains initially picked up by the pollinator (πR). In contrast, d_k increases and then decreases with increases in ρ , with the k th recipient flower receiving the most donor pollen when $\rho = 1/k$ (i.e., $\partial d_k / \partial \rho = 0$ at this value of ρ). Because of these relations, few recipient flowers receive donor pollen when either the pollinator initially removes few donor grains, or a large proportion of the pollen on the pollinator's body is deposited on each stigma. The total number of donor grains reaching recipient stigmas is

$$\begin{aligned} D &= \sum_k \rho\pi R(1 - \rho)^{k-1} \\ &= \pi R \end{aligned} \quad (2)$$

so that this model proposes that all pollen carried away from the donor flower is successfully dispersed (see the appendix to this chapter for the general solution to the sum of a geometric series). Pollen can be lost from this system, but only during removal from the donor flower when the fraction $1 - \pi$ of the R grains displaced from the anthers is not carried away by the pollinator.

Two-Compartment Model: Pollinator Grooming

Unlike the process depicted by the single-compartment model, pollen dispersal by most animals probably involves pollen loss during transport. This loss can occur either because pollen is dislodged from the pollinator's body during groom-

ing (e.g., bees and flies; see Holloway, 1976; Macior, 1967; Michener et al., 1978; Kimsey, 1984; Thomson, 1986; Harder, 1990b) or proboscis coiling (e.g., butterflies and moths; see Levin and Berube, 1972), or because the pollen on the pollinator's body is not mixed and so becomes buried under layers of pollen from more recently visited flowers (e.g., hummingbirds; see Price and Waser, 1982; Lertzman and Gass, 1983; Feinsinger and Busby, 1987). Although the mechanisms involved differ, these processes should generally produce multiple pools of pollen on the pollinator's body. To illustrate how multiple pollen pools affect pollen dispersal, we now develop a model of pollen transport by grooming pollinators following Harder and Wilson (unpublished). For a corresponding model of pollen layering, see Lertzman and Gass (1983) and Harder and Wilson (unpublished).

Grooming generally mixes pollen, as assumed by the single-compartment model; however, if some "exposed" sites are more susceptible to this behavior than other "safe" sites (e.g., see Kimsey, 1984), then the pollen on the pollinator's body will act as two (or more) linked pools (see Fig. 6.1b for a diagram of the modeled pollen dynamics). We assume that both the safe and exposed sites contact a flower's sexual organs during a pollinator visit. In particular, the pollinator removes a total of R pollen grains from each flower, of which the fraction π_s is added to the safe site and π_e is added to the exposed site (a third fraction, $1 - \pi_s - \pi_e$, falls from the pollinator and is lost). Simultaneously, the flower's stigma receives fractions ρ_s and ρ_e of the pollen from other flowers currently on the safe and exposed sites, respectively. As the pollinator flies between flowers, it grooms and displaces a fraction Γ of the pollen from the exposed site. This groomed pollen suffers one of two fates: a fraction γ_s is moved onto the safe site, whereas the remaining fraction $1 - \gamma_s$ is lost from pollination because it either falls from the pollinator or is groomed into pollen-carrying structures.

Now consider pollen from a specific donor flower. Immediately after visiting $k - 1$ recipient flowers, the pollinator carries s_{k-1} and e_{k-1} donor grains on its safe and exposed sites, respectively. As the pollinator flies to the next flower, grooming moves $\Gamma\gamma_s e_{k-1}$ donor grains from the exposed site to the safe site, leaving $(1 - \Gamma)e_{k-1}$ donor grains on the exposed site. Consequently, the k th recipient flower receives

$$d_k = \rho_s(s_{k-1} + \Gamma\gamma_s e_{k-1}) + \rho_e(1 - \Gamma)e_{k-1}$$

pollen grains from the donor flower. Incorporation of definitions for e_{k-1} and s_{k-1} (see Harder and Wilson, unpublished, for derivations) yields

$$d_k = R(\omega\chi^{k-1} + \theta\xi^{k-1}) \quad (3)$$

where

$$\begin{aligned}\omega &\equiv \rho_s \left[\pi_s - \frac{\pi_s \Gamma \gamma_s (1 - \rho_s)}{\rho_s - \rho_c - \Gamma (1 - \rho_s)} \right] \\ \chi &\equiv 1 - \rho_s \\ \theta &\equiv \pi_c \left\{ \rho_c (1 - \Gamma) + \pi_s \Gamma \gamma_s \left[1 + \frac{1 - \rho_s}{\rho_s - \rho_c - \Gamma (1 - \rho_s)} \right] \right\} \\ \xi &\equiv (1 - \Gamma)(1 - \rho_c)\end{aligned}$$

Clearly, pollen dispersal on grooming pollinators is much more complicated than the simple decay process described by the single-compartment model [Equation (1)].

Like the single-compartment model, Equation (3) predicts that the number of pollen grains deposited from a specific donor flower declines with visits to successive recipient flowers. However, grooming quickly depletes donor pollen on the exposed site and therefore causes a faster decline in deposition of donor pollen on the first few recipients. In contrast, distant recipients receive relatively more donor pollen than if pollen were carried in a single compartment, because grooming shifts some exposed pollen to the safe site, so that more pollen passes through the safe site than was originally deposited there by the donor anthers.

Based on the grooming model for pollen dispersal [Equation (3)], the total number of donor grains reaching recipient stigmas is

$$\begin{aligned}D &= R \sum_k (\omega \chi^{k-1} + \theta \xi^{k-1}) \\ &= R \left(\frac{\omega}{1 - \chi} + \frac{\theta}{1 - \xi} \right) \\ &= R \left[\pi_s + \pi_c \frac{\rho_c (1 - \Gamma) + \gamma_s \Gamma}{\rho_c (1 - \Gamma) + \Gamma} \right] \quad (4)\end{aligned}$$

This model therefore proposes that, because of grooming, the fraction

$$\frac{\pi_s \Gamma (1 - \gamma_s)}{(\pi_s + \pi_c) [\rho_c (1 - \Gamma) + \Gamma]}$$

of the $(\pi_s + \pi_c)R$ pollen grains that the pollinator carries away from each flower never reaches stigmas.

The characteristics of total pollen dispersal for both the single- [Equation (2)] and two-compartment models [Equation (4)] were explicitly derived from the donor (male) perspective; however, they also represent the recipient (female) perspective of the total number of pollen grains deposited by each pollinator. This symmetry of male and female perspectives on pollen dispersal can be

demonstrated for the single-compartment model by first identifying the equilibrium number of pollen grains carried by each pollinator. The number of grains carried by a pollinator just before arriving at flower x , L_x , is the sum of the number of pollen grains on the pollinator before visiting flower $x - 1$ that were not deposited on that flower, $(1 - \rho)L_{x-1}$, and the number of grains the pollinator removed and carried away from the preceding flower, πR , or

$$L_x = (1 - \rho)L_{x-1} + \pi R$$

At equilibrium, $L_x = L_{x-1} = L^*$, so that $L^* = \pi R / \rho$. When a pollinator visits a flower, it deposits the fraction ρ of the pollen it carries on the stigma. Consequently, each flower receives $\rho L^* = \pi R$ pollen grains, which equals the number of grains dispersed from each flower [see Equation (2)]. A similar equivalence between male and female perspectives can be demonstrated for the two-compartment model (Harder and Wilson, unpublished). This symmetry between dispersal and deposition should be a general feature (on average) of plants whose flowers function simultaneously as pollen donors and recipients.

The preceding models formalize pollen removal, transport, and deposition as the interaction of parameters that specify the probability of pollen moving from one location to another. Although these parameters describe processes, they also represent specific floral and pollinator characteristics. Both the one- and two-compartment models include parameters that summarize the interaction between the pollinator, the pollen presented in a flower (R and π and its variants), and the flower's stigma (ρ and its variants). These parameters generally depend on the size and placement of the respective sexual organ(s), the size and orientation of the pollinator, and the duration of the pollinator's visit, which in turn depends on reward availability. In addition, the grooming model includes pollinator-specific parameters that summarize the details of grooming behavior (Γ and γ_s).

An Example of the Influences of Floral Characters on Pollen Carryover

The preceding models of animal pollination propose that the pattern of pollen dispersal depends on stamen characteristics of the donor flower and pistil characteristics of recipient flowers. We now illustrate these influences by describing some results of a study of pollen dispersal for *Pontederia cordata*, a heterostylous species (Harder and Barrett, unpublished). Many heterostylous species are particularly well suited to studies of the influences of flower structure on pollen dispersal for two reasons. First, heterostyly involves two (distyly) or three morphs (tristyly) that differ in the placement of anthers and stigmas, so that this unusual reproductive system explicitly involves pronounced intraspecific variation in floral structure. Second, in many species the two or three different anther levels produce pollen of distinctly different sizes (Darwin, 1877; Ganders, 1979; Dulberger, 1992), so that pollen from one morph can be readily identified on the stigmas

of another. This feature enables studies of pollen dispersal between sexual organs at the same level (legitimate pollination) without resorting to manipulative techniques (such as emasculation) that can alter pollen dispersal (see Price and Waser, 1982; Thomson, 1986; Morris et al., 1994; Harder and Wilson, unpublished).

The observations we present involve flowers of the mid-styled morph of *P. cordata*, which produce both short-level anthers (included within the perianth tube) and exerted, long-level anthers. Given this arrangement of anthers, the legitimate recipients for this morph are long-styled plants (for long-level pollen) and short-styled plants (for short-level pollen). Pollen produced by the two anther levels of mid-styled flowers differs significantly in size (long-level pollen, mean \pm SE diameter = 35.6 ± 0.24 μm , $n = 19$; short-level pollen, 18.7 ± 0.07 μm , $n = 19$) and can be readily distinguished from the mid-level pollen of other morphs (long-styled morph, 28.5 ± 0.15 μm , $n = 22$; short-styled morph, 28.0 ± 0.13 μm , $n = 17$; Harder and Barrett, 1993). No morph produces pollen at the same level as its stigma, so that pollen from a donor plant of one morph can be unequivocally identified on the stigma of a legitimate recipient if the pollinator does not visit other plants of the donor's morph before it visits the recipients. Measurement of pollen carryover therefore involved picking donor and recipient inflorescences during early morning before the single-day flowers opened; enclosing these inflorescences to preclude pollinator visits until the flowers opened and anthers dehisced; allowing a clean bumble bee (*Bombus vagans* Smith) to visit a donor inflorescence with 10 flowers followed by up to six recipient inflorescences of a specific legitimate morph; and counting the appropriate pollen on the stigmas of visited recipient flowers. Because bumble bees begin visiting *P. cordata* flowers before anthesis, we could collect clean, but experienced, bees as we picked inflorescences.

Several aspects of pollen carryover are immediately obvious from the results for mid-styled *P. cordata* flowers (Fig. 6.2). First, if a single-compartment model appropriately described pollen carryover for this species, then $\log(\text{pollen deposition} + 1)$ should decline linearly in Fig. 6.2. Instead, deposition declines relatively quickly for initial recipients and then more slowly for later recipients (also see Thomson, 1986; Morris et al., 1994). This pattern is more consistent with a two-compartment model of pollen dispersal [Equation (3)], which is not surprising given that the bees involved groomed frequently while flying between flowers (based on videotaped records of experimental trials; Harder and Barrett, unpublished). Indeed, nonlinear regression indicates that the general form of Equation (3) fits the data for both anther levels better than the single-compartment model (based on the absolute sizes of the error mean squares). The regression estimate of $\chi \equiv 1 - \rho_r$ for Equation (3) implies that approximately 97% of the pollen on safe sites remains there between flower visits (long-level anthers, 96.8%; short-level anthers, 97.4%). In contrast, the estimate of $\xi \equiv (1 - \Gamma)(1 - \rho_r)$ suggests that approximately 81% of the pollen on exposed sites is not displaced between visits (long-level anthers, 81.6%; short-level anthers, 80.5%).

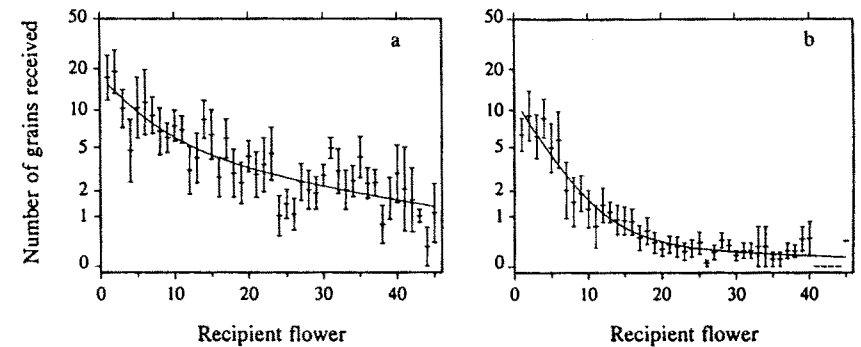


Figure 6.2. Patterns of legitimate pollen deposition (mean \pm SE) for pollen from (a) long- and (b) short-level anthers of mid-styled *Pontederia cordata* flowers. Recipient flowers are identified by the order in which they were visited after the bee left the donor inflorescence. The curves in each panel are the least-squares, nonlinear regression fits of Equation (3). Based on eight trials per anther level.

This apparent imbalance in residence probabilities for the two pollen pools illustrates the need for considering two-compartment models when describing pollen dispersal.

Second, the position of sexual organs in *P. cordata* flowers clearly affects legitimate pollen dispersal. Comparison of Figs. 6.2a and 6.2b indicates that more pollen reaches a given recipient flower from long-level anthers than short-level anthers. For example, deposition declines so rapidly for short-level pollen that flowers beyond the 13th recipient receive fewer than one pollen grain, on average (Fig. 6.2b), whereas more than 45 recipient flowers receive an average of at least one grain from long-level anthers (Fig. 6.2a). This more extensive dispersal of pollen from long-level anthers occurs despite a 4.4-fold advantage in pollen production for short-level anthers in mid-styled flowers. Such a pronounced difference in successful pollen dispersal suggests that the position of sexual organs primarily affects the susceptibility of pollen to grooming, especially the fraction of exposed pollen that is groomed into safe sites (γ_r).

Finally, Fig. 6.2 illustrates considerable variability around the generally declining trend in average pollen receipt. Such variation is a common feature of pollen dispersal (see Lertzman and Gass, 1983; Geber, 1985; Thomson, 1986; Feinsinger and Busby, 1987; Galen and Rotenberry, 1988; Waser, 1988; Wolfe and Barrett, 1989; Robertson, 1992; Stanton et al., 1992) and implies that animal pollination is highly stochastic. Although many aspects of the interaction between pollinators and flowers could produce such variation, stochastic versions of the grooming model [Equation (3)] indicate that most of the recipient-to-recipient variation probably arises during deposition of pollen on stigmas (i.e., variation in ρ_r and ρ_s ; Harder and Wilson, unpublished).

Pollen Dispersal by Multiflowered Inflorescences and Implications for Mating

As outlined above (see "General Influences of Daily Inflorescence Size on Pollination and Mating"), the simultaneous display of more than one flower bears diverse implications for pollen dispersal from the plant's (rather than the flower's) perspective and for mating patterns. In contrast to its role in pollinator attraction, the effects of daily inflorescence size on pollen dispersal and mating have been little studied. To illustrate these effects, we now examine the consequences of pollen carryover for selfing and outcrossing from both theoretical and empirical perspectives. Based on these results, we then construct a cost-benefit model that identifies how the somewhat antagonistic roles of floral display in pollinator attraction and pollen dispersal can be balanced to maximize a plant's fertility.

Expected Frequency of Selfing and Outcrossing

To appreciate qualitatively the mating implications of displaying more than one flower, consider an example (Fig. 6.3) in which the pollinator visits $V=4$ flowers per plant, carries away $D = \pi R = 200$ grains per flower, and deposits 10% of the pollen it carries on each flower ($p = 0.1$) according to the single-compartment model [Equation (1)]. The four bold lines in Fig. 6.3 depict pollen dispersal from the flowers visited on plant 0, whereas the remaining diagonal lines portray pollen received by plant 0 (and 3) and from other plants (four curves per donor). Consideration of pollen receipt by plant 0 (i.e., between the dotted vertical lines) indicates several aspects of mating. First, movement of the pollinator between flowers on the donor plant results in geitonogamous deposition of pollen from the first three flowers visited. Second, each plant receives pollen from seven outcross donors. Third, these donors make disparate pollen contributions to plant 0, with most recently visited plants donating more pollen than more distant plants. This unequal pollen donation is further clarified by considering pollen export from plant 0 to plant 3 (i.e., between the dashed-dotted vertical lines). Fourth, each donor plant contributes many pollen grains to each of several recipients, producing a correlation in the identity of male gametes received by individual stigmas and by different flowers on a plant. Finally, a donor's perspective of pollen dispersal (e.g., following the bold curves) is equivalent to the recipient's perspective (e.g., considering the four flowers on a given plant), in the number of mates and relative intensity of mating with those mates.

To formalize these influences of daily inflorescence size on pollen dispersal and mating parameters, we now set the single-compartment model for individual flowers [Equation (1)] into the whole-plant context. Although the model represents the processes involved in pollen dispersal less completely than multicompartments models, it serves our purpose of illustrating the general pattern of pollen dispersal among plants. The models that follow incorporate several assumptions.

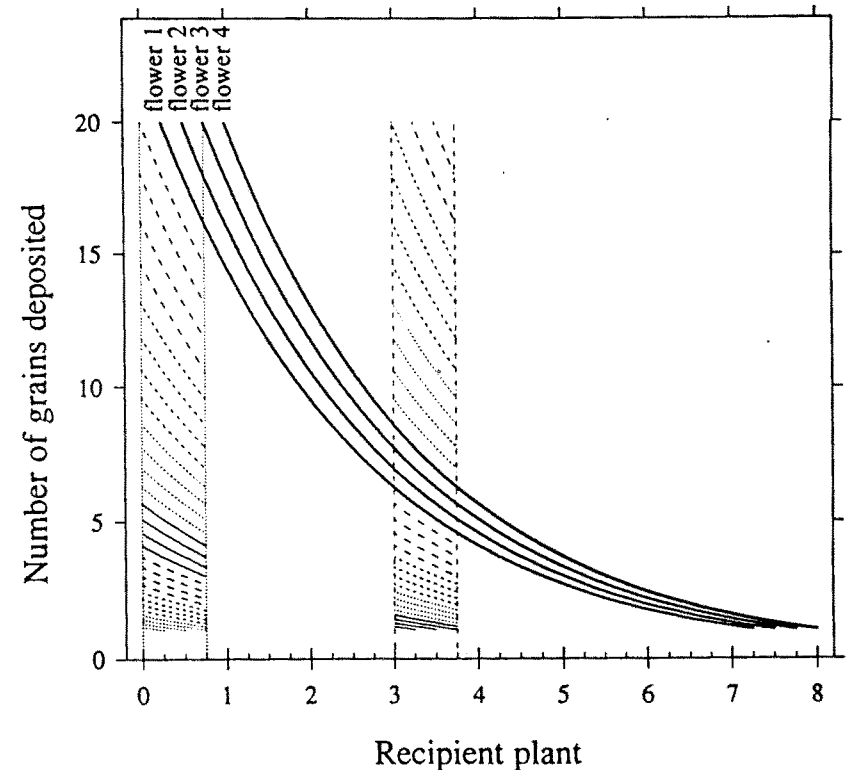


Figure 6.3. Pollen export and import predicted by a single-compartment model of pollen dispersal [Equations (5) and (6)] in which the pollinator carries away 200 pollen grains from each of the four flowers visited per plant and deposits 10% of the pollen it carries on each flower. Large ticks on the abscissa indicate the first flower visited on each plant; small ticks indicate the remaining flowers visited. The bold lines illustrate pollen dispersal from the four flowers visited on plant 0. Vertical blocks depict pollen receipt from all donors by plants 0 and 3. Within these blocks, adjacent lines of the same type represent pollen from flowers on a particular donor plant.

As for Equation (1), we assume that pollen on the pollinator occupies a single, completely mixed pool, each flower contributes $D = \pi R$ grains to this pool, and the stigma of each flower removes a fixed proportion of the pool (p). We additionally assume that the pollinator visits V flowers on each plant (without revisits) and pollen transfer between flowers on the same plant does not differ from that between flowers on different plants. In fact, geitonogamous transfer may be more likely than transfer between plants because bees groom more often while flying between plants (Harder, 1990b). For heuristic purposes, it is easier to

develop this model from the perspective of the recipient plant, rather than the donor plant.

Self-Pollination

Two modes of self-pollination result from the actions of pollinators: movement of pollen between the anthers and stigma of the same flower (facilitated intrafloral selfing) and pollen movement between flowers of the same plant (geitonogamous selfing; Lloyd and Schoen, 1992). In the most general case, each of the V flowers visited on a plant receives I grains through facilitated intrafloral selfing and $V-1$ flowers also receive geitonogamously transferred self-pollen (e.g., see plant 0 in Fig. 6.3). To quantify geitonogamous selfing, consider the j^{th} flower visited on the recipient of interest. Based on Equation (1), flower j receives

$$G_j = \sum_{i=1}^{j-1} \rho D (1-\rho)^{i-1} \\ = D[1 - (1-\rho)^{j-1}]$$

geitonogamous pollen grains (for $j=1$, $G_j=0$). For all flowers visited on the plant of interest, the cumulative number of self-grains received is

$$S = \sum_{j=1}^V (I + G_j) \\ = V(I+D) - \frac{D[1 - (1-\rho)^V]}{\rho} \quad (5)$$

The last term in Equation (5) represents the cumulative number of pollen grains removed from flowers on this plant that are dispersed to other plants. According to Equation (5), self-pollination for the entire plant increases in an accelerating manner with the number of flowers visited by each pollinator.

Cross-Pollination

Now consider the number of outcrossed pollen grains received by the recipient plant from the k^{th} donor plant visited before the recipient. Begin by focusing on the j^{th} flower visited on the recipient and the i^{th} -last flower visited on the donor (e.g., consider plant 0 in Fig. 6.3 as the donor and plant 3 as the recipient). If the pollinator visits V flowers on each plant, then $(k-1)V + i + j - 1$ visits separate these two flowers. Based on Equation (1), flower i donates a total of

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

pollen grains to the recipient plant. Summing over all flowers on the donor plant indicate that

$$O_k = \frac{D[1 - (1-\rho)^V]^2 (1-\rho)^{(k-1)V}}{\rho} \quad (6)$$

grains from donor k reach the recipient plant. This expression for outcrossed pollen dispersal is the product of three terms: the number of donor grains that reach all other plants via a single pollinator, $\sum_k O_k = D[1 - (1-\rho)^V]/\rho$; the fraction of donor pollen remaining on the pollinator just before it visits the recipient, $(1-\rho)^{(k-1)V}$; and the fraction of pollen on the pollinator's body that is deposited on each inflorescence, $1 - (1-\rho)^V$. Equation (6) is merely the original single-compartment model expressed in terms of recipient plants, rather than flowers [note that Equation (6) equals Equation (1) when $V = 1$]. Hence, the incorporation of more than one flower visit per plant does not alter our earlier conclusions for that model concerning the dependence of pollen dispersal on the number of grains carried from the donor by the pollinator ($D = \pi R$) or the deposition fraction ρ . As the complement to self-pollination, outcrossing by the entire plant increases in a decelerating manner with the number of flowers visited per plant.

Mating Parameters

Mating integrates both pollination and postpollination processes. Typically, studies of mating do not distinguish between these processes, but rather assess their cumulative effects, which are summarized by parameters such as s (fraction of selfed seeds), t (fraction of outcrossed seeds), r_s and r_p (correlated selfing and outcrossing, respectively). In this chapter, we are primarily interested in the pollination component of mating (for reviews of postpollination influences, see Lyons et al., 1989; Marshall and Folsom, 1991). Therefore, to avoid confusion with the standard symbols, we define the pollination components of these mating parameters by unique symbols (identified below).

Number of Mates

The number of plants that receive at least one pollen grain from a particular donor plant on a single pollinator, m , is found by setting Equation (6) equal to 1 and solving for $k=m$, which yields

$$m = 1 + \frac{\log \rho - \log D - 2 \log[1 - (1-\rho)^V]}{V \log(1-\rho)} \quad (7)$$

Figure 6.4 illustrates that the number of mates decreases with increases in the deposition fraction, ρ , and the number of flowers visited per plant. In addition,

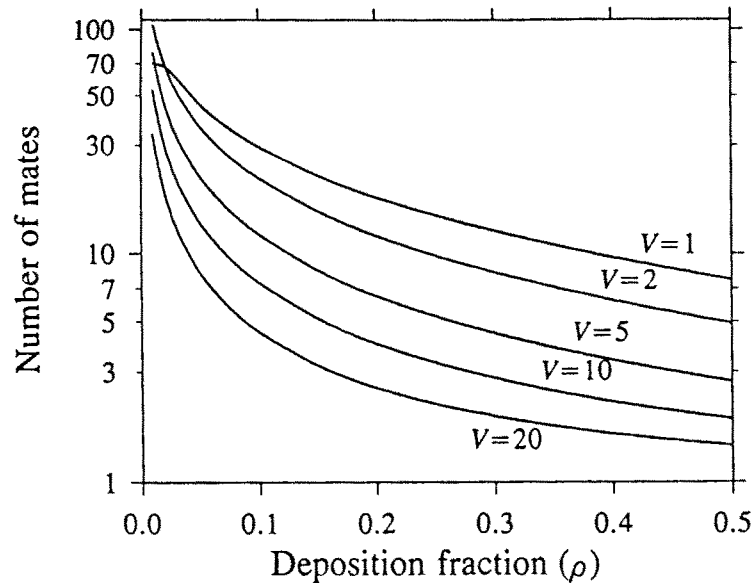


Figure 6.4. Relation of the number of plants contributing pollen to each stigma as a result of pollen dispersal by a single pollinator to the number of flowers visited per plant (V) and the fraction of pollen on the pollinator's body deposited on each flower (ρ). Based on Equation (7), with $D=200$ grains.

the number of mates increases at a decelerating rate with increases in the number of grains exported, D .

Pollination Component of Selfing

Three aspects of self-pollination warrant attention. First, for the j th flower visited on a plant, the fraction of pollen grains that arrive via self-pollination is

$$\begin{aligned}\psi_j &= \frac{I + G_j}{I + D} \\ &= 1 - \frac{D(1 - \rho)^{j-1}}{I + D}\end{aligned}\quad (8)$$

As Barrett et al. (1994) demonstrated, this fraction increases asymptotically with each successive flower visited. Second, the selfing fraction for the entire plant is

$$\begin{aligned}\Psi &= \frac{S}{V(I + D)} \\ &= 1 - \frac{D[1 - (1 - \rho)^V]}{\rho V(I + D)}\end{aligned}\quad (9)$$

which is also the average fraction of self-grains received by the V flowers visited (Barrett et al., 1994, referred to this average as $\bar{\psi}$). According to Equation (9), aspects of pollen dispersal that promote extensive carryover between plants [small ρ , small V [see Fig. 6.5b] or large D] reduce the fraction of self-pollen received by stigmas. In contrast, Ψ is positively related to the intensity of intrafloral selfing I . Third, any self-deposited pollen that would otherwise have been dispersed to other plants represents lost outcrossed pollen (pollen discounting; Holsinger et al., 1984). Equation (9) illustrates that pollen discounting can involve two components: one geitonogamous, the other intrafloral. All geitonogamously deposited pollen is discounted, because it is included in the pool of pollen on the pollinator's body that is destined to be deposited on other flowers (Lloyd, 1992). In contrast, intraflorally deposited pollen is discounted only if it can be considered lost to outcrossing.

Correlated Mating

Ritland (1989) defined correlated mating as the proportion of all possible pairs of seeds that are full sibs. To quantify correlated mating from the perspective of the pollen received by a plant, one simply considers the fraction of all possible pairs of received grains for which both grains are derived from the same donor. If the plant receives N grains, then there are

$$\binom{N}{2} = \frac{N(N-1)}{2}$$

possible pairs of grains. Similarly, if plant k donated n_k of these grains, then $n_k(n_k - 1)/2$ of the pairs of grains will have originated solely from this donor. As a result, the proportion of con-paternal pollen pairs donated by plant k is

$$\frac{n_k(n_k - 1)/2}{N(N-1)/2} = \frac{n_k(n_k - 1)}{N(N-1)}$$

If plant k is also the recipient plant, then this equation estimates correlated self-pollination. Correlated cross-pollination from all m donors that contributed pollen is

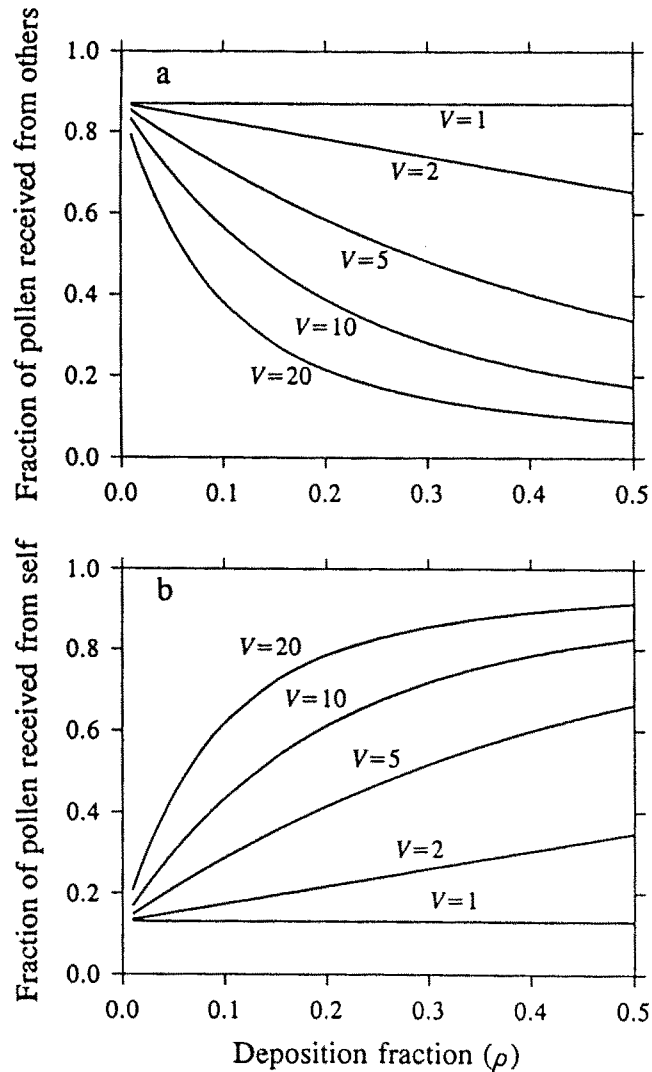


Figure 6.5. Relation of (a) the fraction of outcrossed pollen received and (b) the fraction of self-pollen, ψ , received to the number of flowers visited per plant (V) and the fraction of pollen on the pollinator's body deposited on each flower (ρ). Based on Equation (9), with $D=200$ grains and $I=30$ grains.

$$\frac{\sum_{k=1}^m n_k(n_k - 1)}{N(N - 1)}$$

We can relate correlated self- and cross-pollination to pollen dispersal by recognizing that $N = V(I + D)$, $n_k = S$ [see Equation (5)] for self-pollination, and $n_k = O_k$ [see Equation (6)] for cross-pollination. Hence, correlated self-pollination is

$$\Phi_s = \frac{S(S - 1)}{V(I + D)[V(I + D) - 1]} \quad (10)$$

Because Equation (10) approaches Ψ^2 as the number of self grains increases, correlated selfing is influenced by pollen dispersal in much the same manner as the selfing fraction, Ψ [see Equation (9)], being negatively related to parameters that extend carryover and positively related to the intensity of intrafloral selfing (see Fig. 6.5b).

Correlated cross-pollination is

$$\Phi_o = \frac{\sum_{k=1}^m O_k(O_k - 1)}{V(I + D)[V(I + D) - 1]} \quad (11)$$

which differs from correlated selfing in that it does not depend on dispersal parameters in a simple monotonic fashion (Fig. 6.6). When pollinators visit few flowers per plant, decreases in carryover (large ρ or small D) reduce the number of mates (see Fig. 6.4) and increase the proportion of pollen contributed to those mates that are involved. On the other hand, when pollinators visit more than a few flowers per plant, correlated outcrossing is maximal at intermediate values of the deposition fraction ρ . This peak in correlated outcrossing occurs because low and high values of ρ lead to relatively equitable contributions of pollen per mate, although for very different reasons. Low values of ρ produce relatively flat carryover curves, resulting in many mates each receiving similar contributions of pollen per mate. At high values of ρ , increased pollen discounting resulting from geitonogamous self-pollination removes most donor pollen from the pollinator before it can move to an unrelated plant (see Fig. 6.5b), so that outcrossing entails the relatively flat tail of the carryover curve, which involves little pollen per donor.

Ritland (1989) derived a simple relation between correlated outcrossing and pollen carryover [$\Phi_o = \rho/(2 - \rho)$ in terms of the parameter definitions of this chapter], which he proposed could be applied to estimate ρ once correlated outcrossing had been estimated from electrophoretic data. This relation is a

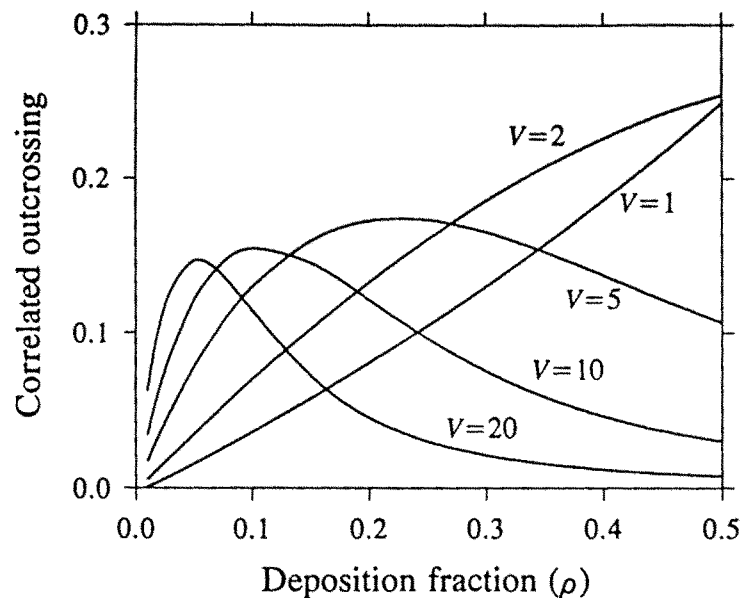


Figure 6.6. Relation of correlated outcrossing to the number of flowers visited per plant (V) and the fraction of pollen on the pollinator's body deposited on each flower (ρ). Based on Equations (5), (6), (10), and (11), with $D=200$ grains and $I=30$ grains.

special case of Equation (11), with $V = 1$, $I = 0$ and large D and m . However, when pollinators visit more than one flower per plant and/or their visits result in intrafloral selfing, the relation of correlated outcrossing and pollen carryover is more complex than Ritland suggested (see Fig. 6.6). Unfortunately, such complexity implies that estimating ρ requires more information than a measure of correlated outcrossing, even if a single-compartment model [Equation (6)] adequately describes pollen dispersal.

Applicability of Model

Even if individual pollinators dispersed pollen according to the single-compartment model, the patterns of mate diversity for flowers, inflorescences, and plants will typically differ from those described in the preceding sections. Repeated pollinator visits complicate mating patterns for individual flowers whenever pollinators follow different flight paths and consequently arrive with pollen from different donors and carry pollen to different recipients. Furthermore, a plant's flowers experience distinct visit chronologies if each pollinator does not visit the same subset of flowers. Because successive pollinator visits to a flower remove different amounts of pollen (Galen and Stanton, 1989; Harder, 1990a; Young

and Stanton, 1990; Klinkhamer et al., 1991; Harder and Wilson, 1994; LeBuhn and Anderson, 1994), flowers with dissimilar histories will contribute different amounts of pollen to the pool on the pollinator's body, so that the relative amount of pollen received from different flowers (and plants) will deviate from the scenario outlined in Fig. 6.3. The extent of such deviation will depend on the schedule and pattern of anthesis within and between inflorescences, the schedules of pollen removal and receipt, inflorescence architecture, and pollinator behavior. Repeated visits will generally disrupt the symmetry of male and female perspectives on pollen dispersal associated with dispersal by individual pollinators. The symmetry of mating (as opposed to pollination) will be further altered by postpollination processes that favor particular pollen donors in determining seed paternity. In spite of these concerns, the following empirical results illustrate that the predicted roles of pollen dispersal in mating are quite robust (also see Barrett et al., 1994).

Observations of the Effects of Daily Inflorescence Size on Pollination and Mating

To examine the diverse influences of daily inflorescence size on pollination and mating, we conducted an experiment involving artificial arrays of *Eichhornia paniculata* (Spreng.) Solms-Laubach (Pontederiaceae). This fully self-compatible, tristylous species produces up to 20 single-day flowers per inflorescence per day. Although the inflorescence is structurally a panicle, it functions as a raceme as only one flower opens per panicle branch on a given day. All plants used in this experiment were long-styled, so that the results are not complicated by heterostyly. The arrays included 35 or 36 plants (depending on the treatments involved) arranged in a 6×6 grid with approximately 30-cm spacings. Experimental plants produced flowers on a single inflorescence and excess flowers were removed from inflorescences to produce 3-, 6-, 9-, or 12-flowered plants. During an individual trial, an array included either all plants of one inflorescence size (pure arrays), or plants of two sizes (pairwise arrays) with the number of plants per treatment adjusted so that both treatments involved the same total number of flowers within the array (i.e., equivalent pollen and ovule production per treatment). The experiment included all four pure arrays and all six possible pairwise arrays, with four replicates per combination.* For several weeks prior to the experiment, we placed *E. paniculata* plants at the array locations so that the resident bees were familiar with this species.

During each experimental trial, we recorded pollinator activity and sampled fruit. An array was set up in the morning before anthesis. Once flowers opened, we observed pollinator behavior for 15 min during each of the first 3 h of

*Replication involved arrays in two locations that were sampled on each of 2 days. Details of the effects of location will be presented elsewhere.

flowering. These observations included counts of the entries and exits to the array by bees [primarily *Bombus vagans* and *B. fervidus* (Fabricius)] and records of the sequence of inflorescence visits (including position in the array and number of flowers visited) by focal bees. Once flowers wilted in late afternoon, one flower from each of the top, middle, and bottom thirds of the inflorescence was marked with paint. Capsules produced by marked flowers were collected once the seeds had ripened (11–12 days) and stored in separate envelopes until the seeds could be counted and assayed electrophoretically.

The plants used in this experiment contained known electrophoretic markers so that we could both count the seeds sired by plants of each inflorescence size and estimate the fraction of selfed seeds and correlated mating. Plants in all arrays were polymorphic for *PGI-2*. In pairwise arrays, plants with one inflorescence size were homozygous for the fast *AAT-3* allele and plants in the competing treatment were homozygous for the slow *AAT-3* allele. Pure arrays included equal numbers of plants with one of these homozygous *AAT-3* genotypes.

Pollinator Preferences

Assessment of pollinators' preferences for particular inflorescence sizes in our array experiment is complicated because the protocol for constructing pairwise arrays resulted in plants with larger inflorescences being less abundant than those with smaller inflorescences. To accommodate this inequality in availability, we calculated the following preference index:

$$\text{Preference} = \ln \left(\frac{\text{odds that the bee visited a large inflorescence}}{\text{odds that any inflorescence was large}} \right)$$

for each focal bee that we observed visit more than 10 inflorescences.† If a bee visits large inflorescences in proportion to their relative abundance, this index equals 0, whereas preference for large inflorescences results in a positive index and preference for small inflorescences results in a negative index.

Based on this index, bees' preferences varied with the ratio of the two inflorescence sizes in an array (Fig. 6.7). For example, the mean preference for 6-flowered plants in arrays with 3- and 6-flowered plants is virtually identical to the mean preference for 12-flowered plants in arrays with 6- and 12-flowered plants (Fig. 6.7, ratio of inflorescence sizes = 2). Trend analysis (Kirk, 1982) of these results indicates that increases in the ratio of inflorescence sizes increased bees' preference for large inflorescences (linear contrast, $F_{1,21} = 31.00$, $P < 0.001$), but reduced the incremental advantage of larger inflorescences (quadratic component of linear+quadratic contrast, $F_{1,21} = 9.04$, $P < 0.01$). These

†The odds of an event is the ratio of the probability of the event occurring to the probability of the event not happening.

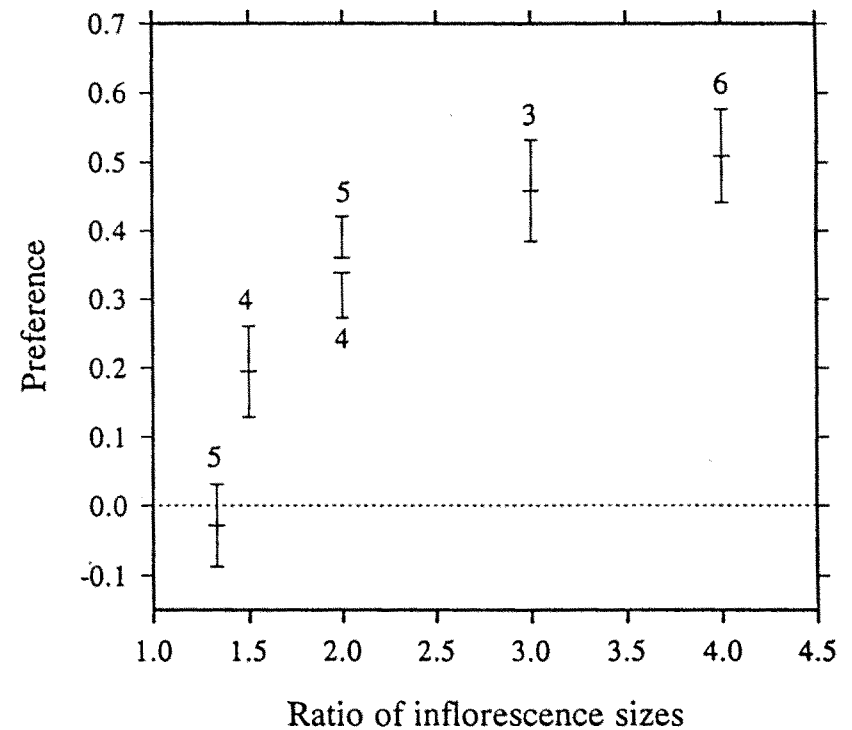


Figure 6.7. Mean (\pm SE) preference by bumble bees for larger inflorescences in pairwise arrays of *Eichhornia paniculata* as affected by the relative sizes of available inflorescences. See text for a definition of preference. The dotted horizontal line indicates indifference. Numbers adjacent to error bars indicate the numbers of bees sampled. For two treatment combinations, one inflorescence size included twice as many flowers as the competing size. Therefore to avoid confusion, the results for three-flowered vs. six-flowered plants do not include a lower error bar, whereas the results for six-flowered vs. 12-flowered plants lack an upper error bar. (After Harder and Barrett 1995).

results imply that flower number per se does not affect a bee's preferences for particular inflorescences; instead, the attractive advantages of producing more flowers arise in competitive situations involving a variation in daily inflorescence size. Indeed, the frequency of bee entries to pure arrays did not vary significantly with inflorescence size (Barrett et al., 1994).

Number of Flowers Visited per Inflorescence

Our assessment of whether the number of flowers visited per inflorescence increases in proportion to the number of open flowers considered the median

number of flowers visited by each bee. Earlier analysis of the pure arrays alone (Barrett et al., 1994) indicated an increasing decelerating relation between daily inflorescence size and the number of flowers visited per pollinator (also see Chapter 7). Further analysis of the complete experiment indicates that a bee's behavior depends on both the number of flowers on the inflorescence being visited and the sizes of other available inflorescences (flower number \times competitor interaction, $F_{9,137}=2.73$, $P<0.01$). In particular, these results indicate that the number of flowers visited increased with inflorescence size, but the greatest increase occurred when competing inflorescences were considerably smaller than the inflorescence being visited (Fig. 6.8). Such context-dependent pollinator behavior greatly complicates the selective influences on pollination, especially as they relate to daily inflorescence size. Regardless of the pollination environment, the increase in the number of flowers visited did not keep pace with increases in inflorescence size, so that bees visited a smaller proportion of the flowers on 12-flowered inflorescences ($1/3$ – $1/2$) than they visited on 3-flowered inflorescences ($2/3$). Overall, the increased attractiveness of large inflorescences

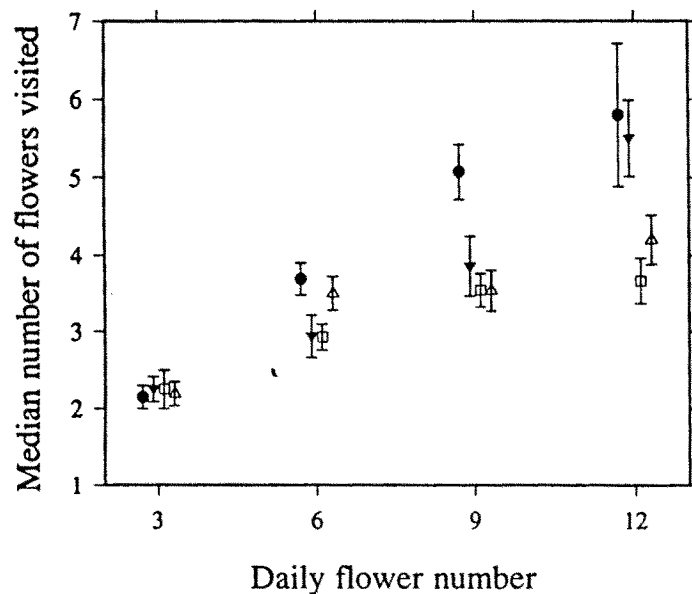


Figure 6.8 Mean (\pm SE) median number of flowers visited per inflorescence by *Bombus fervidus* workers in experimental arrays of *Eichhornia paniculata* in relation to inflorescence size and the size of competing inflorescences (solid circles, 3-flowered competitors; solid downward-pointing triangles, 6-flowered competitors; open squares, 9-flowered competitors; open upward-pointing triangles, 12-flowered competitors). Based on 3–12 bees per treatment combination. (After Harder and Barrett 1995).

counterbalanced the declining proportion of flowers visited per inflorescence per pollinator, so that the cumulative number of visits per flower did not differ significantly among inflorescence sizes ($F_{3,21} = 2.40$, $P > 0.05$, based on an analysis similar to that presented in the "Pollinator preferences" Section).

Seed Production

Overall, seed production in this experiment was pollen-limited, as the average flower produced 56.5 (SE = 1.38, $n = 184$) seeds, whereas a sample of flowers in the pure arrays that received supplemental pollination produced an average of 90 seeds (see Barrett et al., 1994). This pollen limitation probably resulted because the arrays involved only long-styled flowers of a tristylous species, so that anthers and stigmas contacted very different positions on pollinators' bodies. In spite of the preferences by bees for larger inflorescences in competitive situations, flower number per inflorescence did not significantly affect seed set ($P > 0.75$). Consequently, differences in relative fertility (see below) did not arise from a variation in seed production. Furthermore, because seed production was not resource-limited, the mating events recorded by seed genotypes should accurately reflect the pollination component of mating, especially given that for *E. paniculata*, self and intramorph pollen have equivalent pollen-tube growth and siring ability (Cruzan and Barrett, 1993) and this species seldom aborts fertilized seeds (Morgan and Barrett, 1939; Toppings, 1989).

Frequency of Self-Fertilization for Entire Inflorescences

Equation (9) proposes that the cumulative incidence of selfing for all flowers on an inflorescence increases with daily inflorescence size. To test this prediction, we determined the electrophoretic genotypes of three seeds from three flowers for every inflorescence in all arrays. Based on these data, we estimated the total fraction of selfed seeds, s , and the associated standard error (based on 100 bootstrap samples) for each inflorescence size in an array with Ritland's (1990) multilocus selfing rate program. Influences on s were assessed with ANOVA, in which individual estimates of s were weighted by the inverse of their squared standard error.

As predicted, the fraction of selfed seeds increased with daily inflorescence size (Fig. 6.9a; $F_{3,16} = 3.30$, $P < 0.05$). Trend analysis of this effect indicated that almost all the variation in s associated with inflorescence size could be explained by a linear relation ($F_{1,16} = 9.47$, $P < 0.01$). In contrast, Equation (9) describes a decelerating relation; however, consideration of only four inflorescence classes allows little power for assessing this more complex hypothesis. The effects of inflorescence size were not influenced by the size of competing inflorescences in an array ($P > 0.75$ for both the main competition effect and its interaction with inflorescence size of the focal treatment).

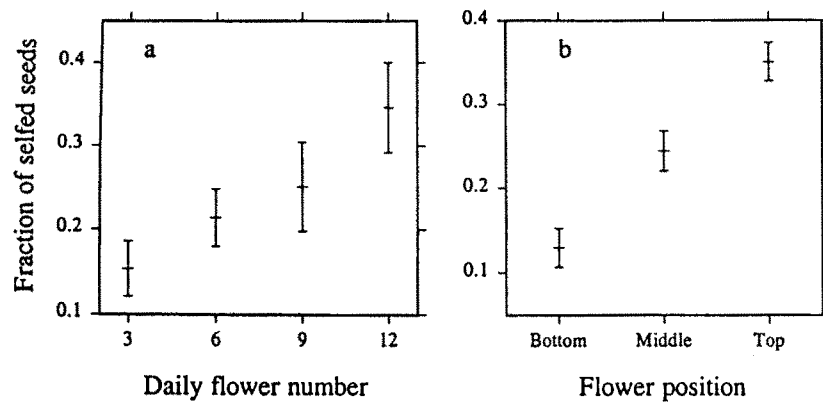


Figure 6.9. Mean (\pm SE) proportion of selfed seeds per inflorescence-size treatment in relation to (a) daily inflorescence size and (b) flower position within the inflorescence for *Eichhornia paniculata* in experimental arrays. Each mean is based on 16 replicates. (After Harder and Barrett 1995).

Frequency of Self-Fertilization and Flower Position

The preceding analysis amalgamates the fraction of selfed seeds for all flowers within an inflorescence; however, Equation (8) proposes that the first flowers visited by a pollinator on an inflorescence should experience less selfing than those visited later in the same bout. The vertical structure of *E. paniculata* inflorescences and the tendency of the bees involved to visit low flowers first and then move upward (Barrett et al., 1994; also see Manning, 1956; Percival and Morgan, 1965; Waddington and Heinrich, 1979; Corbet et al., 1981; Best and Bierzychudek, 1982) implies that the fraction of selfed seeds should increase from bottom to top flowers. To test this prediction, the three flowers involved in the estimates of selfing described in the preceding section were collected from the bottom, middle, and top of the inflorescence, respectively. We could therefore estimate the fraction of selfed seeds with respect to flower position in the ANOVA.

Position within an inflorescence strongly affected a flower's fraction of selfed seeds (Fig. 6.9b; $F_{2,32} = 23.75$, $P < 0.001$). This relation did not vary among inflorescence sizes (position \times size interaction, $F_{6,32} = 0.99$, $P > 0.25$), perhaps because the many bees visiting an inflorescence neither start on the same flower nor visit flowers in the same sequence. In addition, position effects did not vary with the size of competing inflorescences in an array ($P > 0.25$ for all interactions involving position and the size of competing inflorescences).

Correlated Outcrossing

Probably the least intuitive result of our theoretical analysis of the effects of pollen dispersal on mating parameters involved correlated outcrossing. In

particular, given low to moderate pollen carryover, the fraction of seeds that are full sibs via outcrossing increases, peaks, and then decreases with increasing inflorescence size (Fig. 6.6). We tested this prediction with estimates of correlated outcrossing (Ritland, 1990) for the pure arrays. A weighted ANOVA of these data indicates significantly higher correlated outcrossing for 6-flowered plants than for 3-, 9-, or 12-flowered plants (Fig. 6.10); $F_{3,20} = 5.11$, $P < 0.01$). The maximal correlated outcrossing at an intermediate inflorescence size is consistent with Equation (11); however, the similarity of correlated outcrossing for 9- and 12-flower plants is not expected. The remarkable consistency in estimates of correlated outcrossing for 3-, 9-, or 12-flowered plants suggests the occurrence of a lower limit.

Outcrossed Siring Success

Daily inflorescence size could affect the number of seeds that a plant sires by two complementary mechanisms. First, pollen discounting resulting from geitonogamous pollination reduces the amount of pollen available for outcrossing by large inflorescences [see Equation (9)]. Second, the increased pollen removal resulting from a bee visiting more flowers per inflorescence could stimulate more thorough grooming by the bee, thereby increasing the amount of pollen lost during transport (see Harder and Thomson, 1989; Harder, 1990b). As a consequence of

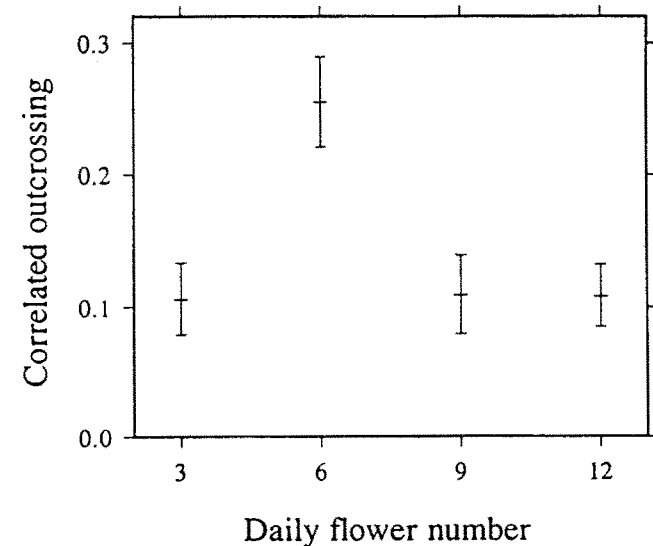


Figure 6.10. Mean (\pm SE) proportion of pairs of seeds that are full sibs via outcrossing in relation to daily inflorescence size for *Eichhornia paniculata* in pure arrays. Each mean is based on 16 replicates.

these mechanisms, large inflorescences should sire a smaller proportion of the outcrossed seeds produced by an array.

Estimation of the proportion of all outcrossed seeds sired by inflorescences of a particular size in pairwise arrays required identification of outcrossed seeds and their paternity. Based on our previous estimates of the fraction of selfed seeds per inflorescence size s (see the "Frequency of Self-Fertilization for Entire Inflorescences" section above and Harder and Barrett, 1995), we estimated the number of outcrossed seeds on inflorescences of size i , $(1-s_i)T_i$, where T_i is the total number of seeds produced by those inflorescences. This *maternal* perspective on outcrossing differs from the *paternal* perspective in that it includes seeds produced by treatment i inflorescences that were sired by treatment j plants, B_i , and excludes seeds sired by treatment i plants on treatment j plants, B_j . These seeds sired by pollen transfer *between* the inflorescence-size treatments (B_i and B_j) could be identified unequivocally because they were heterozygous for *AAT-3*, whereas all parental plants were homozygous at this locus. Given estimates of the number of outcrossed seeds on maternal plants and the numbers of seeds sired by between-treatment pollination, the fraction of outcrossed seeds sired by treatment i plants (outcrossed siring success) is

$$o_i = \frac{(1-s_i)T_i - B_i + B_j}{(1-s_i)T_i + (1-s_j)T_j}$$

All else being equal, the competing inflorescence-size treatments in pairwise arrays should sire the same numbers of seeds, so that $o_i = 0.5$. In contrast, small inflorescences sired a significantly greater proportion of seeds than large inflorescences (paired t -test, $t_{23} = 2.74$, $P < 0.02$; mean \pm SE difference in $o_i = 0.077 \pm 0.028$). Furthermore, outcrossed siring success declined with the frequency of self-fertilization (Fig. 6.11; $\hat{o}_i = 0.573 - 0.254s_i$; $F_{1,22} = 18.43$, $P < 0.001$, $R^2 = 0.239$). These results indicate that the increased geitonogamous selfing that accompanies large inflorescences (Fig. 6.9a) causes significant pollen discounting and thereby reduces siring success through outcrossing (also see Harder and Barrett, 1995). Whether changes in pollen loss associated with pollinator grooming additionally affected outcrossed siring success is less clear, as we did not measure grooming losses.

In this experiment, we used unequal numbers of plants with competing inflorescence sizes so that both inflorescence treatments in a pairwise array were represented by the same total number of flowers. As a result, each size presented equivalent numbers of gametes, but they were deployed differently on individual plants. Such a situation would occur if all plants produced the same numbers of flowers during their entire flowering period (i.e., equal total inflorescence size), but they differed in their daily inflorescence sizes and consequently flowered for different periods. Because few-flowered plants sired more seeds than many-flowered plants, our experimental results imply that individual plants would

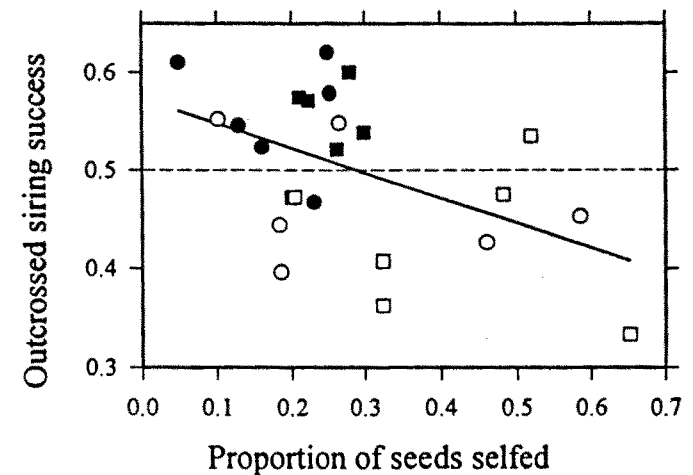


Figure 6.11. Effect of the frequency of self-fertilization, s_i , on relative outcrossed siring success, o_i , in pairwise arrays. The solid line illustrates the regression between o_i and s_i , whereas the dashed line indicates equivalent outcrossed siring success for competing inflorescence-size treatments. This analysis excluded pure arrays because outcrossing is the exact complement of selfing when only one treatment is involved. We also included data for only one inflorescence treatment per array (randomly selected) because the outcrossed siring success of one treatment is the complement of that for the competing treatment in an array. Symbols indicate inflorescence sizes: solid circles, 3 flowers; solid squares, 6 flowers; open circles, 9 flowers; open squares, 12 flowers. (After Harder and Barrett 1995).

maximize their male fertility by presenting few flowers each day (i.e., small daily inflorescence size) and flowering for a protracted period. This conclusion applies specifically to plants with the (unknown) carryover characteristics at play during this experiment: other characteristics can promote more liberal floral display (see the following section).

A Cost-Benefit Analysis of Daily Flower Production

During the course of this chapter, we have outlined the variety of influences of daily inflorescence size on pollination and mating. The benefits of simultaneously displaying many flowers primarily relate to enhanced attraction of pollinators to the entire plant. Increased inflorescence size could additionally reduce self-pollination for species that self as flowers wilt (delayed selfing; Lloyd and Schoen, 1992) if increased attractiveness resulted in more visits per flower, leaving less pollen in exhausted flowers (Schoen and Dubuc, 1990). However, large floral displays often result in fewer visits per flower (Schmitt, 1983; Geber, 1985; Andersson, 1988; Klinkhamer et al., 1989; Robertson, 1992) and promote pollen

discounting through geitonogamous pollen transfer, which can greatly reduce siring success if self-pollination triggers processes that compromise a pollen grain's chance of producing a viable seed (e.g., self-incompatibility, higher abortion, or inbreeding depression). The conflicting benefits and costs of exposing many flowers to individual pollinators imply that some intermediate daily flower production maximizes a plant's fertility (see Klinkhamer and de Jong, 1993).

To assess how pollination and mating influence optimal daily inflorescence size, consider a plant that produces F flowers during its flowering season (i.e., $F \equiv$ total inflorescence size), of which f are displayed each day. If we assume that pollen receipt limits seed production by all flowers, then the symmetry of female and male perspectives on mating assures that every seed has only one mother and one father. We also assume power functions between daily inflorescence size, the number of pollinator visits to the inflorescence per day (i.e., $n = af^b$) and the number of flowers visited by each pollinator (i.e., $V = f^c$).^{*} In the absence of pollinator-induced intrafloral selfing, the number of seeds produced as a result of geitonogamous pollination is proportional to

$$(1 - \delta) \left[VD - \frac{D[1 - (1 - \rho)^V]}{\rho} \right]$$

where δ is the reduction in the success of self pollen relative to outcrossed pollen [see Equation (5)]. Correspondingly, genetic contributions through outcrossing are proportional to

$$\frac{D[1 - (1 - \rho)^V]}{\rho}$$

Half of these contributions comprise the plant's own outcrossed seeds and half involve seeds sired on other plants by the focal plant. In sum, a plant makes genetic contributions to

$$\frac{D}{\rho} \left\{ (1 - \delta)\rho V + \delta [1 - (1 - \rho)^V] \right\}$$

seeds as a result of a single pollinator visit.

To quantify the fertility resulting from all visits on a given day, we must specify some pattern of pollen removal during successive pollinator visits (i.e., define D for each of the n pollinators) and sum the resulting fertilities. For

^{*}de Jong et al.'s (1992) model of daily inflorescence size differs from the model presented here in assuming that each pollinator visits all exposed flowers. Our model describes this situation if $c=1$.

simplicity, we assume that each pollinator removes a fixed proportion, P , of the pollen remaining in a flower, so that the total number of viable seeds per day, T , is proportional to

$$T \propto \frac{h(fPA_0)^g [1 - (1 - P)^{gn}]}{\rho [1 - (1 - P)^g]} \left\{ (1 - \delta)\rho V + \delta [1 - (1 - \rho)^V] \right\}$$

where A_0 is the initial pollen production per flower, and h and g describe the relation between pollen removal and pollen dispersal (D) per pollinator [see Harder and Thomson, 1989; Harder and Wilson (1994) assess the assumption of constant proportional removal].[†] Finally, a plant that produces f flowers per day until it has produced F flowers blooms for F/f days, so that the total number of viable matings per inflorescence is $M = FT/f$, or

$$M \propto \frac{Ff^{g-1}h(PA_0)^g [1 - (1 - P)^{gn}]}{\rho [1 - (1 - P)^g]} \left\{ (1 - \delta)\rho V + \delta [1 - (1 - \rho)^V] \right\} \quad (12)$$

The optimal daily inflorescence size[‡] is found by setting the partial derivative of Equation (12) with respect to f equal to 0 and solving for f^* , yielding

$$0 = (g - 1)[1 - (1 - P)^{gn}] \{ (1 - \delta)\rho V + \delta [1 - (1 - \rho)^V] \} \\ - bgn(1 - P)^{gn} \ln(1 - P) \{ (1 - \delta)\rho V + \delta [1 - (1 - \rho)^V] \} \\ + cV[1 - (1 - P)^{gn}] \{ (1 - \delta)\rho - \delta(1 - \rho)^V \ln(1 - \rho) \} \quad (13)$$

Although Equation (13) lacks a direct solution (recall that n and V are functions of f), this expression exposes several conclusions. First, F , A_0 , and h have canceled, so that according to this model, the optimal daily inflorescence size does not depend on total inflorescence size (as long as $F > f$), pollen production per flower, or the proportionality constant of the relation between pollen removal and subsequent dispersal. Rather, f^* depends on the details of pollinator attraction and the number of flowers visited per pollinator (i.e., a , b , and c), pollen removal per pollinator, P , pollen carryover, ρ , and the relative fertility of selfed pollen, δ . Second, the optimal daily inflorescence size does not generally maximize the number of pollinator visits per flower ($nV/f = af^{b+c-1}$). In particular, although the total number of matings is maximized at some intermediate flower production,

[†]de Jong et al.'s (1992) model of daily inflorescence size differs from the model presented here in not incorporating a decelerating relation between pollen removal and dispersal (see Harder and Thomson, 1989). Our model describes this situation if $g=1$.

[‡]This model of optimal daily inflorescence size explicitly maximizes absolute fitness. The corresponding ESS model that maximizes fitness of a rare phenotype with daily inflorescence size of f_1 flowers relative to the fitness of the predominant phenotype with f_0 flowers (i.e., $w = M_1/M_0$) identifies the same optimal flower production.

visits per flower is maximal for either a single flower, when $b + c < 1$, or infinite flowers, when $b + c > 1$. Third, when selfing is not detrimental, so that geitonogamous pollen discounting affects only the seed genotypes (i.e., $\delta = 0$), Equation (13) reduces to

$$0 = (c + g - 1)[1 - (1 - P)^{gn}] - bgn(1 - P)^{gn} \ln(1 - P)$$

which does not include ρ . Hence, in the absence of a cost of selfing, the details of pollen carryover do not affect optimal daily inflorescence size.

Numerical solution of Equation (13) for specific parameter values reveal further details of the influences on optimal daily inflorescence size. When self-pollen has a low probability of producing viable seeds (i.e., $\delta \rightarrow 1$), the consequences of pollen discounting are severe for many-flowered inflorescences. As a result, the optimal daily inflorescence size is smaller for given levels of pollen removal P , and carryover, ρ , than when selfing is not detrimental (i.e., $\delta \rightarrow 0$; compare Figs. 6.12a and b). Large inflorescences can be optimal even with a large cost of selfing given two conditions (see Fig. 6.12). First, if relatively little of the pollen carried by a pollinator is deposited on each flower (i.e., extensive carryover; small ρ), then comparatively little pollen is deposited geitonogamously, resulting in limited pollen discounting. Second, restricted pollen removal during

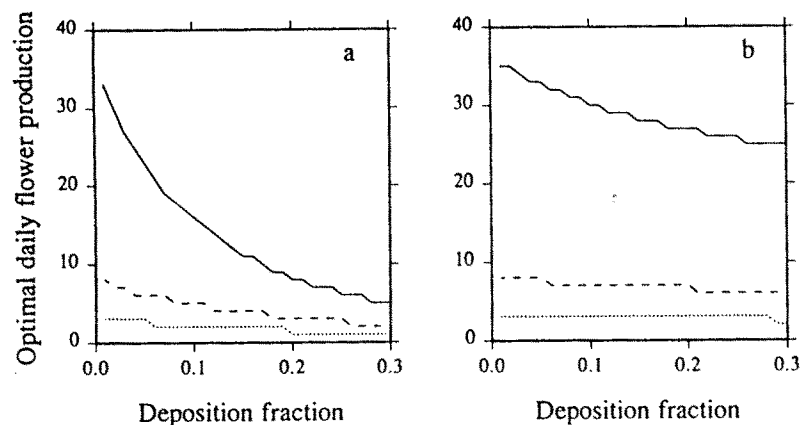


Figure 6.12. Relation of optimal daily inflorescence size to the proportion of remaining pollen removed per pollinator (P), the fraction of pollen on the pollinator's body deposited on each flower (deposition fraction ρ), and the reduction in the value of a selfed seed relative to an outcrossed seed (δ). The different lines in each panel relate the optimal flower display when $P = 0.1$ (solid lines), 0.2 (dashed lines), or 0.3 (dotted lines). Panel (a) depicts complete inbreeding depression (i.e., $\delta = 1$), whereas for panel (b) self-pollen is 80% as likely to fertilize seeds as outcrossed pollen (i.e., $\delta = 0.2$). Based on numerical solutions to Equation (13), with $a = 5$, $b = 0.5$, $c = 0.4$, and $g = 0.3$.

individual pollinator visits (i.e., small P) ameliorates the diminishing returns (i.e., $g < 1$) associated with animal pollination and results in greater cumulative pollen dispersal as long as many pollinators are involved (Harder and Thomson, 1989; Harder and Wilson 1994). Hence, large flower displays are feasible with restricted pollen removal in spite of pollen discounting because the increased pollen dispersal resulting from attracting more pollinators exceeds discounting losses. This conclusion further implies that the geitonogamous self-pollination associated with the optimal inflorescence size (see Fig. 6.13b) represents a cost of maximizing pollen dispersal and receipt, rather than a selected feature in its own right (also see Lloyd, 1992).

Schoen and Dubuc (1990) previously derived a model of inflorescence size and number that complements our model in several ways. Schoen and Dubuc concentrated on total inflorescence size and incorporated the resource costs of producing more flowers. Their model implicitly assumed a direct correlation between total F and daily inflorescence size f to incorporate pollination influences.* In contrast, our model assumes that total flower production has been resolved (i.e., F is fixed) and identifies the pollination and mating influences on optimal daily flower production. In doing so, we assumed that resource constraints are less relevant to optimal daily inflorescence size. Any such constraint will further increase the costs of simultaneously displaying many flowers and reduce the optimal daily inflorescence size.

Evolutionary Implications

Pollination affects fertility through both the diversity of outcrossed matings and the relative frequency of selfing. The functional linkage between pollination and mating implies that selection that increases average fertility will often result in correlated evolution of floral characters and mating systems. As a result, some combinations of floral characters and mating systems should be more prevalent than others, such as the relation between intrafloral self-pollination and the proximity of anthers and stigmas within a flower (Breese, 1959; Rick et al., 1978; Ennos, 1981; Schoen, 1982; Thomson and Stratton, 1985; Barrett and Shore, 1987; Barrett and Husband, 1990; Murcia, 1990; Robertson and Lloyd, 1991; also see Chapter 14). We believe that many more associations between floral characters and mating systems remain to be discovered.

The consequences of pollen discounting promise to be a particularly rich area for future analyses of the interaction between pollination and mating. As defined by Holsinger et al. (1984), pollen discounting describes the extent to which self-

*The applicability of this assumption apparently varies between species as Harder and Cruzan (1990) found intraspecific correlations ranging from 0.150–0.676 for nine legume species (significant correlations for seven species) and from 0.229–0.900 for eight ericad species (significant correlations for seven species).

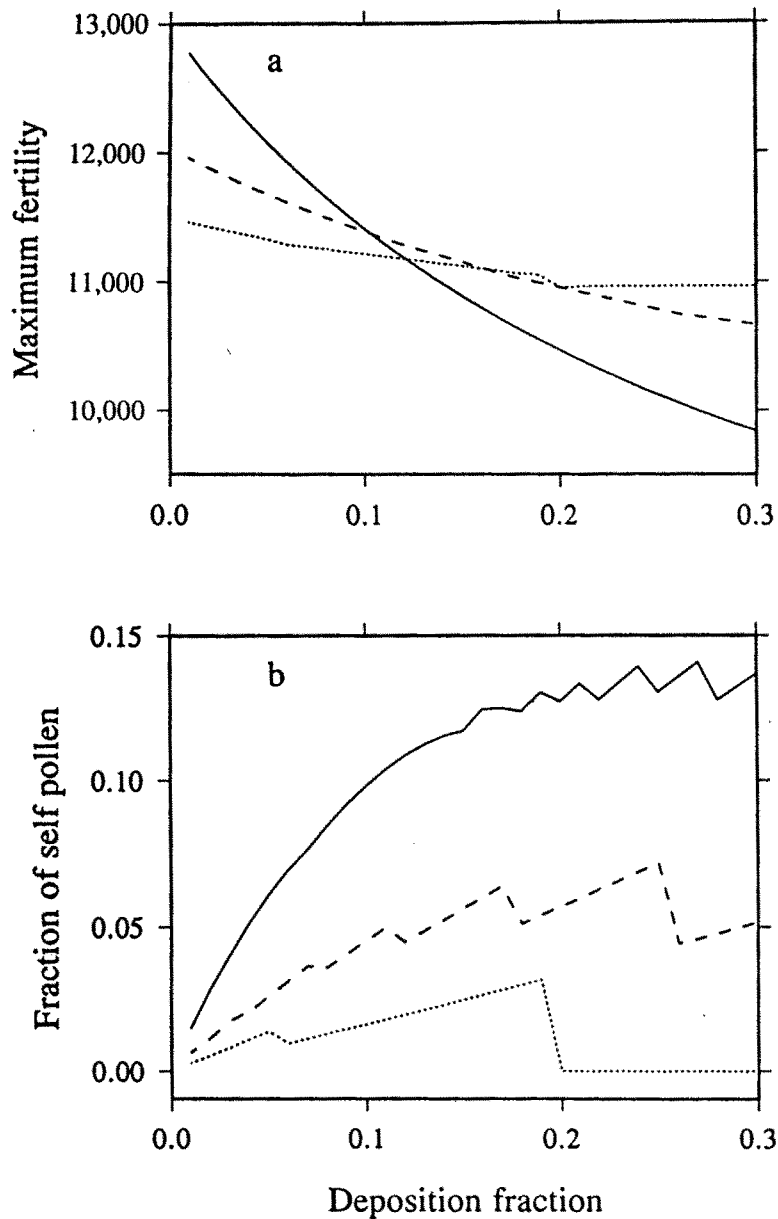


Figure 6.13. Relation of (a) maximum fertility and (b) the fraction of self-pollen, Ψ , to the proportion of remaining pollen removed per pollinator (P) and the fraction of pollen on the pollinator's body deposited on each flower (deposition fraction ρ). The different lines relate the optimal flower display when $P=0.1$ (solid lines), 0.2 (dashed lines), or 0.3 (dotted lines). Based on Equations (12) and (9), respectively, with P^* determined by numerical solution of Equation (12), for $A_0=50,000$ grains, $a=5$, $b=0.5$, $c=0.4$, $\delta=1$, $g=0.3$, $h=1$, and $F=150$ flowers.

pollination reduces the number of pollen grains involved in cross-pollination. Such reduction affects the evolution of self-fertilization because discounting lessens the advantage of facultatively selfing individuals (Nagylaki, 1976; Wells, 1979; Holsinger et al., 1984; Holsinger, 1988), which, in the absence of discounting, contribute an average of three gene copies to the next generation (two through each selfed seed and one through paternal contribution to outcrossed seed) for every two copies contributed by outcrossing individuals (Fisher, 1941). Our model of daily inflorescence size identified additional implications of pollen discounting when discounting is coupled with postpollination processes that compromise the fertilization success of self-pollen. Indeed, it is helpful to recognize that the consequences of pollen discounting for male fertility range from increased average homozygosity of offspring when selfed and outcrossed offspring are equally successful ("cross-discounting") to the absolute failure of self-pollen with complete incompatibility or inbreeding depression ("fatal discounting"; also see de Jong et al., 1992, 1993). Because the complete absence of inbreeding depression is probably uncommon (see Jarne and Charlesworth, 1993), theoretical analyses of the evolution of selfing should realistically incorporate the interaction between pollen discounting and postpollination losses (Uyenoyama et al., 1993). Furthermore, whenever postpollination processes discriminate against self-pollen or selfed zygotes, pollen discounting reduces male fertility and should strongly influence floral evolution.

Whether pollen discounting represents a significant loss of outcrossed-mating opportunities, and hence is a significant process in plant ecology, genetics, and evolution, has been questioned (Lloyd, 1992; Holsinger and Thomson, 1994). This doubt arises from the observation that "(p)ollen grains are much more numerous than ovules, and . . . (c)onsequently, an increase in self-fertilization can often be achieved with a minimal effect on the outcrossing pollen pool" (Lloyd, 1992, p. 372; also see Holsinger and Thomson, 1994). Although it may be true that pollen grains are plentiful and individually inexpensive, the important consideration is not production, but the number of mating opportunities. Given that <1% of a plant's pollen typically reaches stigmas (e.g., Levin and Berube, 1972; Galen and Stanton, 1989; Harder and Thomson, 1989; Wolfe and Barrett, 1989; Young and Stanton, 1990), the relevant question is not "how much pollen is involved in selfing?", but rather "how much of the pollen that would otherwise reach other plants is involved in selfing?" Because geitonogamous pollen transfer is functionally equivalent to interplant transfer (Fægri and van der Pijl, 1979; Lloyd and Schoen, 1992), especially when pollinators fly between flowers in the inflorescence, geitonogamous selfing will generally draw pollen directly from the restricted pollen pool carried by the pollinator that is destined for deposition on other flowers. Hence, although intrafloral self-pollination may result in little pollen discounting, particularly for herkogamous species, geitonogamous self-pollination probably commonly limits opportunities for outcrossing (Lloyd, 1992). It is particularly striking that the two studies reporting little evidence of

discounting involved single-flowered plants (Rausher et al., 1993; Holsinger and Thomson 1994), whereas demonstrations of extensive pollen discounting involved plants that simultaneously display many flowers (Ritland, 1991; Holsinger, 1992; Kohn and Barrett, 1994; this study).

Mating Patterns and the Evolution of Floral Design and Display

Our analysis of the mating consequences of pollination emphasizes that the open flowers in an inflorescence do not act independently during a plant's interaction with individual pollinators. Given the fundamental role of geitonogamous pollination in determining the incidence of self-pollination and pollen discounting (Figs. 6.5, 6.9, and 6.11), the flower cannot be considered the operational unit of either male or female function for animal-pollinated plants; rather, this role belongs to the entire floral display. This conclusion influences ecological, genetic, and evolutionary interpretations of the design of individual flowers, because the mating consequences of characters that affect the pattern of pollen dispersal depend on how many flowers a pollinator visits on the same plant. Hence, complete assessment of the functional significance of flower shape and size, the size and position of sexual organs, reward characteristics, and the schedules of male and female function requires consideration of their effects on the entire plant's mating success.

In our model of daily inflorescence size, we treated pollen removal and carryover as evolutionarily fixed; however, the details of both processes depend on various floral characteristics (e.g., anther and stigma placement, corolla shape; pollen-dispensing mechanisms, stigma size and structure) that are undoubtedly subject to selection (e.g., Campbell, 1989; Galen, 1989; Johnston, 1991). Because of the functional correlations between such characters, the evolution of daily inflorescence size likely occurs in concert with the modification of flower form. In general, large inflorescences should accompany restricted pollen removal and extensive carryover, because fertility is globally maximized under these conditions (see Fig. 6.13a). Alternatively, if either restricted removal or extensive carryover is not evolutionarily feasible, then fertility is maximized by exposing few flowers at any time (also see de Jong et al., 1992; Robertson, 1992). In spite of the expectation of functional correlations between floral and inflorescence characters, few studies have assessed their occurrence through comparative interspecific analysis (although see Harder and Cruzan, 1990). Such studies are necessary to appreciate fully the evolutionary impact of interactions between floral design and display. Unfortunately, our current limited understanding of the role of specific floral characters in pollen carryover precludes the formulation of detailed hypotheses.

Although geitonogamous pollen discounting can constrain the evolutionary opportunities for plants to enhance pollinator attraction through larger displays, several floral adaptations relieve this constraint. The simplest means of increasing

display size without paying discounting costs involves the incorporation of flowers that neither donate nor receive pollen in the display. Some species accomplish this by producing sterile flowers at the periphery of the inflorescence (e.g., many Asteraceae with radiate capitula, some *Viburnum* spp., *Hydrangea* spp.), whereas other species maintain flowers for protracted periods after their primary sexual roles are complete. Such flowers contribute to long-distance signaling of the location of floral rewards but, because they typically differ in morphology or color from rewarding flowers, they indicate which flowers are unrewarding to attracted pollinators (Gori, 1983; Cruzan et al., 1988; Weiss, 1991).

Dichogamy, the temporal separation of male and female function within individual flowers (reviewed by Lloyd and Webb, 1986), also enables large floral displays with reduced discounting costs, particularly when inflorescence structure and pollinator behavior interact so that individual pollinators visit female-phase flowers before male-phase flowers. Dichogamy has traditionally been interpreted as a mechanism discouraging self-fertilization (see Chapter 14), even though many dichogamous species also exhibit self-incompatibility (reviewed by Lloyd and Webb, 1986), and dichogamy is equally prevalent among self-compatible and self-incompatible species (Bertin and Newman, 1993). As a complement to this "antiselfing" explanation, Lloyd and Webb (1986) proposed that dichogamy promotes outcrossing by reducing interference between male and female organs in individual flowers. Although dichogamy necessarily eliminates intrafloral interference, we believe that it probably plays a more important role in reducing the interference *between* flowers on the same inflorescence that results in geitonogamous pollen discounting. By reducing within-plant discounting, dichogamy allows plants to disperse more pollen to other plants on individual pollinators, while allowing the simultaneous presentation of many flowers (some female and the remainder male) to attract more pollinators. Because both self-incompatible species and self-compatible species subject to inbreeding depression suffer from the lost mating opportunities associated with geitonogamous pollination, the incidence of a "cross-promotion" mechanism such as dichogamy should not vary with the occurrence of physiological antiselfing mechanisms, as Bertin and Newman's (1993) survey indicates.

Heterostyly probably represents the epitome of floral mechanisms that promote outcrossing by restricting pollen discounting. For heterostylous species, anthers and stigmas occupy dissimilar positions within flowers on individual plants, but other plants in the population produce the reciprocal arrangement(s) of sexual organs (see Barrett, 1992a). In a monomorphic species, the extreme herkogamy exhibited by an individual plant of a heterostylous species would preclude efficient pollen transfer. For example, our array experiments with the long-styled morph of *Eichhornia paniculata* resulted in pollen limitation of seed production even though the plants were frequently visited, with often three or four bees simultaneously visiting an array of 36 plants (also see Kohn and Barrett, 1992). Although such herkogamy clearly limits pollen transfer within morphs, our demonstration

of the extensive pollen carryover between morphs of *Pontederia cordata* (Fig. 6.2) illustrates that the reciprocal arrangement of sexual organs on different plants promotes crossing between morphs (also see Lloyd and Webb, 1992). Such cross-pollination undoubtedly results because stigmas of one morph receive comparatively little of the pollen produced by noncorresponding anther levels, thereby enabling more pollen to reside on the pollinator until it visits a legitimate recipient. Like dichogamy, heterostyly enables large flower displays; however, because heterostyly limits pollen dispersal between inflorescences of the same morph [pollen wastage; see Bateman, 1952; Baker, 1954 (cited in Lloyd and Webb, 1992); Yeo, 1975; Glover and Barrett, 1986], it will reduce pollen discounting for clonal species more effectively than would dichogamy, which functions only within inflorescences.

The fertility advantages of inflorescence-level mechanisms that enhance pollinator attraction while limiting pollen discounting are clearly illustrated by reinterpretation of Podolsky's (1992, 1993) analysis of andromonoecy in *Besleria triflora*. Podolsky (1992) demonstrated that daily inflorescence size positively affected the frequency of hummingbird visits per inflorescence and per flower. Because seed production by *B. triflora* is pollen-limited, such increased attraction directly benefits female fertility. Indeed, Podolsky (1992) concluded "... that enhanced male function is not necessary for the evolution of andromonoecy" (p. 2259) because although staminate flowers increase attractiveness, they "... were less effective than perfect flowers both in the length of time pollen was presented and in the quantity of pollen dispersed per visit" (p. 2258). However, this emphasis on individual flowers ignores the fact that andromonoecy is an inflorescence characteristic, not a floral characteristic. Consequently, the relevant question is "Does the presence of staminate flowers benefit the *plant's* male fertility more than the presence of additional perfect flowers?" Such an advantage for male fertility seems likely for *B. triflora* because donor flowers dispersed more pollen to recipient flowers when a hummingbird visited intervening staminate flowers than when the intervening flowers were perfect and therefore acted as recipients (Podolsky, 1992). Hence, although the addition of staminate or perfect flowers equally increases an inflorescence's attractiveness (Podolsky, 1992), the addition of staminate flowers would promote male fertility more effectively because the addition of perfect flowers increases geitonogamous pollination and *B. triflora* suffers strong inbreeding depression (see Podolsky, 1992). In summary, andromonoecy in *B. triflora* probably increases both male and female fertility, so that this breeding system exemplifies an additional mechanism enabling increased pollinator attraction without aggravating pollen discounting.

Pollinator Service and Mating-System Evolution

Unsatisfactory pollinator service is frequently invoked as a mechanism promoting diverse evolutionary outcomes, ranging from the evolution of self-fertilization

(Henslow, 1879; Müller, 1883; Baker, 1955; recent studies reviewed by Barrett, 1988; also see Chapter 14) to shifts between outcrossing breeding systems (e.g., Ganders, 1978; Delph, 1990; Weller and Sakai, 1990; Barrett, 1992b). The seeming paradox of a single mechanism producing very different outcomes (Barrett et al., 1992) actually arises because pollinator service can be unsatisfactory for two reasons. *Insufficient* pollinator service arises whenever plants receive too few visits, resulting in pollen limitation of seed production, regardless of quality. In contrast, *inferior* pollinator service results in sufficient pollination to overcome pollen limitation, but the pattern of pollen dispersal limits opportunities for gametophytic competition and mate choice to produce the highest-quality offspring. These two sources of unsatisfactory pollinator service favor very different evolutionary responses by both floral characters and mating systems, so that assessing their importance requires different experimental approaches.

Insufficient pollination results because pollinators are either rare or have been attracted by other plant species, and it promotes characters that result in self-pollination and the assurance of seed production. In general, the amount of reproductive assurance required to favor the spread of a selfing variant depends on both the mode of self-pollination (i.e., how and when self-pollination occurs) and frequency of pollinator visits (Lloyd, 1992). The few studies that have examined both the fraction of selfed seeds and pollinator activity in different populations reported correlations that support the reproductive assurance hypothesis (Rick et al., 1978; Wyatt, 1986; Piper et al., 1986; Husband and Barrett, 1992). However, no studies have examined how the fraction of selfed seeds varies with the number of visits received by individual flowers and/or plants, let alone considered how this relation depends on the mode of selfing. Such experiments are central to testing the reproductive assurance hypothesis.

Inferior pollination and its effects on offspring quality arise because pollinators either disperse pollen poorly among conspecific plants (intraspecific inefficiency) or they do not consistently visit a single species, thereby transferring pollen between species (interspecific pollination). Intraspecific inefficiency results because inadequate pollen carryover or excessive pollen discounting (either intrafloreal or geitonogamous) increases self-pollination and limits the diversity of potential mates. In contrast, interspecific pollination results in receipt of foreign pollen that can interfere with fertilization by conspecific pollen (Thomson et al., 1981; Armbruster and Herzig, 1984; Stucky, 1985; Waser and Fugate, 1986; Harder et al., 1993) and the loss on foreign stigmas of pollen that would otherwise have reached conspecific stigmas (Campbell, 1985; Campbell and Motten, 1985; Feinsinger and Busby, 1987; Feinsinger et al., 1988; Feinsinger and Tiebout, 1991). Like intraspecific inefficiency, interspecific pollination can increase the fraction of selfed seed; however, selfing increases indirectly with interspecific pollination because outcross pollen is intercepted by heterospecific stigmas, thereby diminishing its abundance on conspecific stigmas relative to self-pollen. To our knowledge, no studies to date have examined whether the fraction of

selfed seeds varies either among types of pollinators (although see Chapter 14) or with the relative abundance of other plant species that share the same pollinators. Analysis of evolutionary changes in breeding systems would benefit from such information.

Jarne and Charlesworth (1993) drew attention to a phenomenon that provides our final illustration of the interaction of evolutionary influences on floral and mating-system characteristics. They presented observations of "... large and showy flowers found in the arctic ... (and) a high incidence of self-incompatibility at high altitudes in Patagonia, where insect visitation is low" (p. 454) as examples contradicting the reproductive assurance hypothesis. However, reproductive assurance is only a reasonable evolutionary option if the inbreeding depression that accompanies increased selfing in an originally outcrossing population diminishes over generations as recessive deleterious alleles are purged from the population (see Barrett and Charlesworth, 1991). Such evolutionary reduction of inbreeding depression may be more difficult in rigorous environments, such as those at high latitudes and altitudes, where traits that would be neutral or mildly disadvantageous in more benign conditions become detrimental [see Dudash (1990) for an example of environmental effects on inbreeding depression]. Hence, we propose that an interaction between environmental conditions and the intensity of inbreeding depression constrains the evolution of selfing in stressful environments and favors floral traits that promote outcrossing. This example provides further indication that the outcome of mating (who has mated with whom) cannot be logically separated from the ecological and physiological processes that determine how two specific gametes come to form a zygote (also see Richards, 1986; Lloyd and Schoen, 1992; Lloyd, 1992; Waser, 1993). Given that the ecology and evolution of plant fertility integrate the processes and outcome of mating, we expect that the isolation of pollination and mating-system biology that has characterized studies of plant reproduction during much of the twentieth century will not be sustained.

Appendix: Sum of a Geometric Series

Repeatedly in this chapter, we require the sum of a geometric series, such as

$$S = \sum_{i=1}^n ax^{i-1} \\ = a + ax + ax^2 + ax^3 + \dots + ax^{n-1} \quad (\text{A.1})$$

The solution to this sum can be found by multiplying Equation (A.1) by x

$$xS = ax + ax^2 + ax^3 + \dots + ax^{n-1} + ax^n \quad (\text{A.2})$$

subtracting Equation (A.2) from Equation (A.1)

$$S - Sx = a - ax^n$$

and solving for S , yielding

$$S = \frac{a(1 - x^n)}{1 - x} \quad (\text{A.3})$$

If $-1 < x < 1$ and Equation (A.1) involves an indefinite series (i.e., $n = \infty$), then the x^n term in Equation (A.3) equals zero.

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