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POLLEN DISCOUNTING IN *ERYTHRONIUM GRANDIFLORUM*: MASS-ACTION ESTIMATES FROM POLLEN TRANSFER DYNAMICS

KENT E. HOLSINGER* AND JAMES D. THOMSON†

*Department of Ecology and Evolutionary Biology, U-43, University of Connecticut, Storrs, Connecticut 06269-3043; †Department of Ecology & Evolution, State University of New York, Stony Brook, New York 11794 and Rocky Mountain Biological Laboratory, P.O. Box 519, Crested Butte, Colorado 81224

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Abstract.—Pollen discounting, a reduction in success as an outcross pollen parent as a result of selfing, can reduce or eliminate the reproductive advantage commonly attributed to selfing. Previous estimates of pollen discounting have been based on segregation analysis of progeny from open-pollinated plants. Using data from *Erythronium grandiflorum,* we illustrate how direct measures of pollen transfer can be used to estimate discounting rates, and we discuss the relationship between absolute discounting rates measured in this way and relative discounting rates measured through segregation analysis. Only about 0.4% of the pollen removed from anthers in *E. grandiflorum* is used in selfing, and only a little more (0.5%) is delivered to the stigmas of other plants. Using these estimates in the framework of a mass-action model suggests that the success rate of self-pollen is almost 80 times that of outcross pollen. Thus, variants increasing the discounting rate would appear to have a substantial reproductive advantage in *E. grandiflorum.* Pollen discounting cannot explain the maintenance of an outcrossed mating system in *E. grandiflorum,* and it may also fail in other plants in which a large proportion of the pollen produced never reaches a receptive stigma.

Models of mating system evolution in seed plants commonly assume that self-fertilization requires negligible amounts of pollen (see, e.g., Fisher 1941; Maynard Smith 1977, 1978; Lande and Schemske 1985). If that is so, increased selfing need not be accompanied by a decrease in the amount of pollen donated to outcross stigmas. This assumption is responsible for the 50% reproductive advantage attributed to selfers in these models (Maynard Smith 1978; Holsinger 1988, 1992). It is also responsible for the oft-quoted result that natural selection will favor increased rates of selfing if the fitness of selfed progeny is more than half that of outcrossed progeny and will favor decreased rates of selfing if the fitness of selfed progeny is less than half that of outcrossed progeny (see, e.g., Kimura 1959; Lloyd 1979; Lande and Schemske 1985). In fact, the existence of this threshold led Lande and Schemske to their controversial suggestions that there is disruptive selection on plant mating systems and that plants with mixed mating systems are evolutionary transients (Lande and Schemske 1985; Schemske and Lande 1985).

Other models require instead that any increase in the selfing rate be accompanied by a decrease in the amount of pollen donated to outcross stigmas (Nagylaki

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1976; Wells 1979; Charlesworth 1980; Feldman and Christiansen 1984; Holsinger et al. 1984). When such pollen discounting is included, both the 50% reproductive advantage associated with selfing and the fitness threshold that determines whether selfing or outcrossing is favored may be reduced or eliminated. Although the possible significance of pollen discounting has been recognized for over a decade, few estimates of its magnitude are available (Ritland 1991; Holsinger 1992; Rausher et al. 1993). Those that are available are based on segregation analysis of progeny from naturally pollinated plants, but pollen discounting may be analyzed in another way.

Detailed studies of pollen transfer in the bee-pollinated lily *Erythronium grandiflorum* (Thomson and Stratton 1985; Harder and Thomson 1989; Thomson and Thomson 1989), for example, allow us to make estimates of pollen discounting from observed patterns of pollen deposition. In these studies, observation of bees, visual pollen markers, and electronic particle counting were used to determine how many grains are contained in anthers when a bee visits a flower, how many of those grains are taken away by the bee, how many of the removed grains are deposited on the flower's own stigma (*E. grandiflorum* most often produces only one flower in a season; J. D. Thomson, unpublished data), and how many were delivered to the stigmas of other plants. We use the data from these studies to estimate rates of pollen discounting in *E. grandiflorum* and to discuss the relationship between measures of discounting derived from pollen transfer data and those derived from segregation analysis. We also present a simple modification of the mass-action model for plant mating systems (Holsinger 1991) that provides additional insight into the evolutionary dynamics of the mating system in *E. grandiflorum*.

MEASURES OF POLLEN DISCOUNTING

Pollen Discounting and Pollen Transfer

Pollen discounting refers to the reduction in success as an outcross pollen parent associated with selfing. The discounting rate is usually defined as "the fraction of the pollen produced that is removed from the outcross pollen pool as a result of selfing" (Uyenoyama et al. 1993, p. 337). For clarity, we refer to the discounting rate defined in this way as the *absolute* discounting rate, just as absolute viability is often defined in analyses of natural selection as the fraction of zygotes produced that survive to reproductive age.

For measures of absolute discounting rates, direct measurements of the amount of pollen removed from anthers and the amount of pollen used in selfing are required. If there are R pollen grains removed from the anthers of a plant and D of them are deposited on that plant's stigmas, then an estimate for the absolute discounting rate is simply D/R. If there are differences among genotypes in the amount of pollen removed from anthers, then the discounting rate of genotype A_iA_j is the mean of D/R for all plants with that genotype.

Genetic approaches for estimating the discounting rate depend on the observation that if genotypes differ in their discounting rate, allele frequencies in the pollen will differ from those in the population as a whole. In fact, the difference in these frequencies is responsible for the dramatic impact that pollen discounting may have on mating system evolution (Nagylaki 1976; Charlesworth 1980; Holsinger et al. 1984). If the absolute discounting rate of genotype A_iA_j is δ_{ij} and each genotype produces equal amounts of pollen, then the frequency of allele A_1 in the outcross pollen pool is

$$p_m = \frac{(1 - \delta_{11})x_{11} + (1 - \delta_{12})x_{12}/2}{(1 - \delta_{11})x_{11} + (1 - \delta_{12})x_{12} + (1 - \delta_{22})x_{22}}, \tag{1}$$

where x_{ij} is the frequency of genotype A_iA_j in the population (and we assume a one-locus, two-allele polymorphism). Notice that p_m is unchanged if we divide both the numerator and denominator of equation (1) by $1 - \delta_{11}$ or $1 - \delta_{12}$ or $1 - \delta_{22}$. Only the ratios of the $1 - \delta_{ij}$ affect p_m. Just as it is *relative* differences in viability that determine genotypic responses to selection, it is *relative* differences in discounting rates that determine genotypic representation in the pollen pool. Thus, genetic approaches provide estimates of discounting rates *relative* to one another.

Not only do genetic methods provide a measure of relative rather than absolute discounting rates, they do not distinguish between differences among genotypes in the *proportion* of pollen devoted to self-pollination (the absolute discounting rate) and differences among genotypes in the *amount* of pollen produced. Equation (1) assumes that all genotypes produce the same amount of pollen. If the amount of pollen produced differs among genotypes, however, equation (1) becomes

$$p_m = \frac{(1 - \delta_{11})f_{11}x_{11} + (1 - \delta_{12})f_{12}x_{12}/2}{(1 - \delta_{11})f_{11}x_{11} + (1 - \delta_{12})f_{12}x_{12} + (1 - \delta_{22})f_{22}x_{22}}, \tag{2}$$

where f_{ij} is the pollen fertility of genotype A_iA_j. Notice that the allele frequency in the outcross pollen pool may differ from that in the population even if all genotypes devote the same proportion of pollen to selfing (i.e., if $\delta_{ij} = \delta^*$ for all i and j). Specifically, genotypes with higher than average pollen production will be overrepresented, and those with lower than average pollen production will be underrepresented in the outcross pollen pool. Thus, relative pollen discounting rates measured by genetic methods may reflect differences among genotypes either in the proportion of pollen devoted to selfing (absolute discounting rates) or in the amount of pollen produced (pollen fertility).

Pollen Discounting and Mating System Dynamics

The evolutionary dynamics of a locus that alters the mating system are determined, in part, by the functional relationship between discounting rates and selfing rates. Specifically, genotypes with a higher selfing rate have a transmission advantage if and only if

$$(1 - \sigma_h)/(1 - \sigma_l) < (1 - \delta_h)/(1 - \delta_l), \tag{3}$$

where σ is the selfing rate and h refers to the genotype with the higher selfing rate and l to the genotype with the lower selfing rate (Nagylaki 1976; Holsinger et al. 1984, eq. [7]). Equation (3) implies that more highly selfing genotypes have a transmission advantage only if their representation in the outcross pollen pool, which is proportional to $1 - \delta_h$, is reduced less than their representation in the pool of outcross ovules, which is proportional to $1 - \sigma_h$. Neither the absolute nor the relative discounting rates alone determine whether selfers have a transmission advantage. Rather, the relationship between them and their corresponding selfing rates determines whether selfers have a transmission advantage. In plants with a discrete mating system polymorphism (e.g., *Mimulus guttatus/micranthus:* Ritland 1991; *Ipomoea purpurea:* Rausher et al. 1993), it may be possible to make genetic estimates of selfing rates and discounting rates separately for each of the genotypes. Then the functional relationship between them could be empirically established. It does not matter that relative rather than absolute discounting rates are measured in these studies, because discounting rates enter equation (3) only in the ratio $(1 - \delta_h)/(1 - \delta_l)$. Similarly, the evolutionary dynamics are unaffected by whether the differential representation of genotypes in the pollen pool is a result of differences among them in absolute discounting rates or in pollen fertility.

An alternative to this direct approach to determining the relationship between selfing and discounting rates is to assume a particular model for the functional relationship between selfing and discounting rates, based on some understanding of pollination dynamics, and to estimate the parameters of this model. In species with a discrete mating system polymorphism, it may then be possible to use a genetic approach to estimate absolute discounting rates and other model parameters. For example, progeny segregation data have been used (Holsinger 1992) to estimate absolute discounting rates and other parameters of the mass-action model (Holsinger 1991) in *Senecio vulgaris*. This model is one example of how the functional relationship between selfing rates and discounting rates might be modeled, but more complicated models that incorporate features more accurately reflecting the behavior of biotic pollinators are easily imagined.

Unlike species with a mating system polymorphism, species like *Erythronium grandiflorum* show no obvious genotypic differences in outcrossing rate, and absolute discounting rates can be related to selfing rates *only* if we assume a particular model for the functional relationship between selfing and discounting rates and estimate the parameters of that model. Only then can observations of the amount of pollen removed from anthers, the amount deposited on self-stigmas, and the amount deposited on the stigmas of other plants be used to infer the fate of mating system variants that differ in their absolute discounting rate, because differences in the absolute discounting rate lead to predictable differences in the selfing rate as well. In the following sections we describe a simple modification of the mass-action model presented elsewhere (Holsinger 1991) and illustrate how data on pollen transfer can be used to estimate the absolute discounting rate and the success rate of outcross pollen. We also show how to use these data and data on the relative competitive abilities of self- and outcross pollen to interpret the evolutionary dynamics in the context of this model.

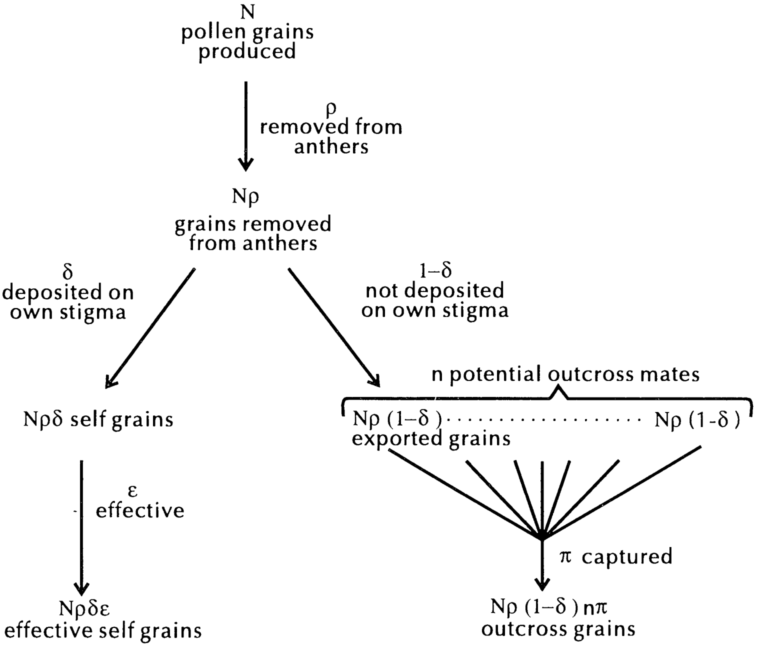

FIG. 1.—A schematic diagram illustrating how to calculate self- and outcross pollen loads from pollen transfer data. Notice that, of N grains produced, $N\ \rho\delta\epsilon$ participate in self-fertilization, and $N\ \rho(1 - \delta)n\pi$ participate in outcross fertilization. The remainder, $N(1 - \rho) + N\ \rho\delta(1 - \epsilon) + N\ \rho(1 - \delta)(1 - n\pi)$, are wasted.

MATERIAL AND METHODS

The Model

Figure 1 presents a straightforward modification of the mass-action model (Holsinger 1991). The only new parameter in the model is ρ, the fraction of pollen pollinators removed from anthers. Here δ is the fraction of pollen removed from anthers that is deposited on a stigma of the plant producing it (i.e., it is the absolute pollen discounting rate). Pollen that is removed from anthers but not used in selfing is pollen potentially available for outcrossing. Most of this pollen is wasted. Also, π is the fraction of the potential outcross pollen captured by the stigma of an individual plant, and n is the average number of plants from which a plant can expect to receive pollen (or to which it can expect to donate pollen). Notice that $n\pi$ is a measure of the success rate of outcross pollen. It is equal to the fraction of pollen grains removed from a plant that eventually reach a stigma, and $1 - n\pi$ is the fraction of the potential outcross pollen that is wasted.

Lloyd (1979) distinguished three possible modes of self-fertilization. In *prior* self-fertilization some fraction of the available ovules are self-fertilized before there is any opportunity for outcrossing. In *delayed* self-fertilization some or all of the ovules remaining unfertilized after outcrossing are self-fertilized. In *competing* self-fertilization self- and outcross pollen compete for access to ovules. If a plant exhibits competing selfing, we can use the mass-action assumption (Holsinger 1991) to calculate the selfing rate, σ, among that plant's ovules. It is simply the proportion of self-pollen on stigmas, weighted by a factor (ϵ) expressing the relative competitive ability of self- and outcross pollen in siring ovules. Specifically,

$$\sigma = \frac{\delta\epsilon}{\delta\epsilon + (1 - \delta)n\pi} \tag{4}$$

(cf. eq. [1] of Holsinger 1991). Notice that if we hold δ and ϵ constant, σ decreases monotonically as the number of potential outcross mates increases. Similarly, if genotypes differ in the amount of pollen used for selfing, the selfing rate of each genotype will increase as the frequency of the genotype that uses the most pollen for selfing increases. In short, this approach to defining a relationship between rates of pollen removal and rates of selfing predicts that selfing rates will be both density- and frequency-dependent—a prediction for which there is some empirical support (Holsinger 1991, 1992).

A bit of algebra suffices to show that the dynamics of this model are identical to that of the basic mass-action model. In particular, a selfing variant will invade an outcrossing population if and only if

$$1 - \delta > n\pi/\epsilon, \tag{5}$$

and the evolutionarily stable discounting rate, δ^*, is given by

$$\delta^* = \frac{1 - n\pi/\epsilon}{2 - n\pi/\epsilon}. \tag{6}$$

Experimental Protocols

In the vicinity of the Rocky Mountain Biological Laboratory in the West Elk Mountains of western Colorado, the glacier lily (*Erythronium grandiflorum* Pursh; Liliaceae) displays a pollen-color dimorphism. Although some populations are monomorphic for yellow pollen, more often one finds a low frequency of red-pollen plants. Because red grains are easily distinguished from yellow grains on stigmas, the dimorphism provides a natural marker for tracing pollen movement (Thomson and Stratton 1985; Thomson and Thomson 1989). The data analyzed here come from an earlier study (Thomson and Thomson 1989), which should be consulted for methodological details.

Briefly, the researchers (Thomson and Thomson 1989) estimated the pollen content of red-pollen flowers from a previously determined regression between anther length and pollen content. They transported these donor flowers into monomorphic yellow populations, allowed the anthers to dehisce undisturbed, then obtained a flower visit from one of the bumblebee queens that are the principal pollinators. They followed the bee as she left the donor, marking the next 40 or so flowers visited. The stigmas of all visited flowers, including the donor, were harvested and the number of red grains counted on each. They also carefully harvested the anthers of the donor into a vial of 70% ethanol and counted the number of grains remaining with an Elzone particle counter. From these data we can calculate the number of grains presented to the pollinator, the number removed from the anthers by the visit, the number deposited on the donor's (self) stigma, and the number deposited on the stigmas of other plants (total outcross deposition). The data are given in table 1 of that study (Thomson and Thomson 1989), although for the present article, the two visits by *Bombus nevadensis* are omitted from the analysis. As explained by those writers (Thomson and Thomson 1989), this bee's altitudinal range does not overlap that of *E. grandiflorum,* and the data obtained from these two visits appear anomalous.

Estimating Model Parameters

Our first objective is to use data on the pattern of pollen transfer to measure the absolute discounting rate (δ) and to estimate the fraction of pollen grains removed from a plant that eventually reach a stigma ($n\pi$). Let P_i be the number of pollen grains produced by the ith plant in this sample, R_i the number of pollen grains removed from the anthers of this plant, S_i the number of pollen grains deposited on that plant's stigma, and D_i the number of pollen grains deposited on the stigmas of other plants. Then an estimate for δ_i, the discounting rate of the ith plant, is

$$\delta_i = \frac{S_i}{R_i}, \tag{7}$$

and our estimate for δ is simply the mean of the δ_i. Similarly, an estimate for $n\pi_i$, the success rate of potential outcross pollen from the ith plant, is

$$n\pi_i = \frac{D_i}{R_i - S_i}, \tag{8}$$

and our estimate for $n\pi$ is the mean of the $n\pi_i$.

To estimate ϵ we used unpublished data (kindly provided by L. Rigney; see also Rigney et al. 1993) on the fraction of selfed seed produced when equal amounts of self- and outcross pollen are placed on a stigma. Sixteen progeny from each of 67 crosses were scored. If p_i is the proportion of selfed progeny among the progeny of the ith maternal plant, then

$$p_i = \frac{\epsilon_i}{\epsilon_i + 1}, \tag{9}$$

where ϵ_i is the relative competitive ability of selfed pollen on the ith maternal parent. Thus, an estimate for ϵ_i is

$$\epsilon_i = \frac{p_i}{1 - p_i}. \tag{10}$$

We can estimate ϵ in the population by $\hat{p}/(1 - \hat{p})$, where $\hat{p}$ is the mean of the p_i. Although this estimate of ϵ is biased, because the expectation of a ratio is not equal to the ratio of expectations, the invariance property of maximum-likelihood estimators (Mood et al. 1974) guarantees that it is a maximum-likelihood estimate

TABLE 1

MATING SYSTEM PARAMETERS IN *ERYTHRONIUM GRANDIFLORUM*

Parameter	Estimate (95% Confidence Limits)
Discounting rate ($\hat{\delta}$)	.00406 (.00293–.00554)
Success rate of self-pollen ($\hat{\epsilon}$)	.471 (.308–.726)
Success rate of outcross pollen ($\widehat{n\pi}$)	.00546 (.00437–.00700)

for ϵ. Furthermore, its bias is small for moderate to large sample sizes (Stuart and Ord 1987).

To place confidence limits on $\hat{\delta}$, $\hat{\epsilon}$, and $\widehat{n\pi}$, the population averages for the respective parameters, we used bootstrapping (Efron 1982), simultaneously resampling the data vectors from which δ_i and $n\pi_i$ were estimated and preserving the covariance structure present in the data. If ϵ is primarily an expression of inbreeding depression, we might expect it to be negatively correlated with δ, because higher values of δ are associated with higher rates of selfing and (presumably) lower levels of inbreeding depression. Unfortunately, different plants were used in the pollen transfer experiments and the pollen competition experiments, so we are unable to address this possible source of covariance in the data. Because ϵ was estimated from a completely separate set of experiments, it was resampled independently of δ and $n\pi$. Comparison of the confidence intervals reported here with those obtained from a bootstrapping procedure that did not preserve the covariance structure and with those obtained from a normal distribution resampling procedure (using the observed means and variances, but setting all covariances to zero) revealed only minor differences. Because the reported confidence limits reported are robust under several different statistical assumptions, it is reasonable to conclude that they are accurate, even though they are based on only 23 pollinator visits.

RESULTS

The amount of pollen that *Erythronium grandiflorum* uses in selfing is extremely small. We estimate that only about 0.4% of the pollen removed from anthers of a flower is deposited on the stigma of the same flower; that is, the absolute discounting rate (δ) is 0.004 (table 1). Similarly, an extremely small fraction of the pollen produced actually participates in outcrossing. Of the pollen that leaves the plant on which it was produced, only about 0.5% reaches the stigmas of other plants; that is, $n\pi$ is 0.005 (table 1; Thomson and Thomson 1989). In short, nearly 99% of the pollen removed from a plant's anthers makes no direct contribution to reproductive success. Furthermore, self-pollen is less than half as likely to fertilize an ovule as outcross pollen with which it competes ($\epsilon = 0.47$; table 1). In control crosses with equal amounts of self- and outcross pollen, only 32% of the progeny result from self-fertilization.

These estimates may provide insight into the evolutionary dynamics of the mating system in *E. grandiflorum*, but only if we can use them to make inferences about the functional relationship between selfing and pollen discounting. The mass-action approach provides one way in which this can be done, providing that self- and outcross pollen grains compete equally for access to ovules. Fortunately, competing self-fertilization does appear to be a reasonable assumption in *E. grandiflorum.*

Self-pollination in *E. grandiflorum* apparently occurs only when flowers are visited. Autogamous fruit set by plants caged to exclude pollinators is rare and apparently restricted to flowers with extraordinarily recurved stigmas. More typically, the pendant flower position and downward-facing stigma act to prevent passive self-pollination. Bee visits, on the other hand, always result in some self-pollination. As a result, self-pollen and outcross pollen tend to be deposited simultaneously, and competing self-fertilization is probably the rule. Thus, we can use our estimates for δ (the absolute discounting rate), ϵ (the success rate of self-pollen), and $n\pi$ (the success rate of outcross pollen) in the mass-action model outlined above to specify the functional relationship between selfing and discounting rates and to determine whether variants that increase the selfing rate have a reproductive advantage.

The mass-action model we used predicts that the selfing rate will increase monotonically with the proportion of pollen devoted to selfing (the absolute discounting rate). Thus, determining whether variants that increase the selfing rate are favored is equivalent to determining whether variants that increase the discounting rate are favored. An increase in the discounting rate will be favored only if additional reproductive success through self-pollen more than compensates for reduced reproductive success through outcross pollen (Holsinger 1992; cf. eq. [3]). In a population with low absolute discounting rates, like *E. grandiflorum,* a variant that increases the discounting rate is favored when the success rate of outcross pollen ($n\pi$) is less than that of self-pollen (ϵ), provided that the relationship in equation (5) is still satisfied. Since ϵ is almost 80 times greater than $n\pi$ (0.471 vs. 0.00546), according to our estimates, we conclude that variants increasing the discounting rate would have a reproductive advantage in these populations. In fact, the evolutionarily stable rate of self-pollen deposition in this population would appear to be nearly 50% if we ignore the possible effects of inbreeding depression.

DISCUSSION

We have shown how data on the dynamics of pollen transport can be used to understand some important aspects of mating system evolution in plants. By analyzing the fate of pollen removed from anthers, we determined the amount of pollen that is actually used in selfing and the success rates of both self- and outcross pollen. Our analysis showed that only about 0.4% of the pollen removed from anthers of *Erythronium grandiflorum* is used in selfing. Only a little more, about 0.5%, is used for outcrossing. Nearly all the pollen removed from the anthers, about 99%, never reaches any stigmas (Thomson and Thomson 1989). More importantly, interpreting these estimates in a mass-action context suggests that a pollen grain deposited on the stigma of the plant producing it is almost 80 times more likely to fertilize an ovule than one removed from the plant by a pollinator, even though self-pollen is less than half as likely to fertilize an ovule as outcross pollen with which it competes. Thus, variants able to increase the proportion of pollen deposited on their own stigmas would appear to have a large reproductive advantage, because additional reproductive success through self-pollen more than makes up for reduced reproductive success through outcross pollen.

The observation that selfing is associated with a large reproductive advantage may seem unsurprising, but it does stand in marked contrast to the results of two studies in which discounting rates were genetically estimated (Ritland 1991; Holsinger 1992). In both of these studies, one in *Mimulus guttatus/micranthus* and the other in *Senecio vulgaris,* high rates of selfing were associated with very high rates of pollen discounting. On the other hand, genetic techniques failed to detect pollen discounting in *Ipomoea purpurea,* which is only moderately selfing (Rausher et al. 1993). It would be intriguing if the low rates estimated in *E. grandiflorum* and *I. purpurea* are characteristic of outcrossing species and those with moderate selfing rates, while the high rates estimated in *M. guttatus/micranthus* and *S. vulgaris* are typical of predominantly selfing species. Clearly, this is an important problem that requires further study in other experimental systems. Studies that combine genetic estimates with those derived from pollen transfer may be particularly informative, because they will also allow us to distinguish differences among individuals in absolute discounting rates from differences in the amount of pollen produced.

In addition to the data presented here and the comparable data of Rausher et al. (1993) on *Ipomoea,* there are other reasons to think that discounting rates may be small in species with low to moderate selfing rates. In biotically pollinated plants, for example, depositing some self-pollen grains will often take away only a tiny fraction of the grains that would otherwise adhere to a pollinator, with most of the grains that do leave the plant being groomed off or otherwise lost before they reach another stigma. In *E. grandiflorum* the chance that a pollen grain removed by a pollinator will reach the stigma of another plant is only about 0.5%. In *Raphanus sativus* the figure is even lower, about 0.06% (Young and Stanton 1990). Thus, the mechanics of pollen transfer are such that there is little reason to expect a trade-off between self- and outcross pollen deposition (i.e., pollen discounting) in biotically pollinated plants. In fact, self-deposition and outcross deposition are positively correlated to *E. grandiflorum* (Pearson's $r = 0.56$, $N = 23$, $P < .01$), not negatively correlated as we would expect if discounting were important. We would not be surprised if this were a common pattern in outcrossing plants with biotic pollinators (in spite of arguments to the contrary; Holsinger 1988).

One striking aspect of our results is the discrepancy between the evolutionarily stable rate of discounting predicted by the mass-action model (nearly 50%) and the low rate of discounting actually observed (0.5%). If we ignore the possibility that our estimation technique is in some way unreliable or highly biased, the implications of our results are that a selfing variant (i.e., a variant with a higher rate of pollen discounting) would have a large reproductive advantage. Why have such variants not spread through the population? There are two possible reasons: The presumed reproductive advantage of selfers does not exist, and inbreeding depression is sufficient to prevent the spread of selfing variants.

The analysis we presented ignores the effect of inbreeding depression, except insofar as it affects the proportion of selfed seed produced. It does not incorporate preferential abortion of selfed seed, nor does it incorporate inbreeding depression that acts at other stages in the life cycle. Nevertheless, it is possible to calculate that inbreeding depression on the order of 50% is necessary to prevent the spread of selfing variants if our calculation of the reproductive advantage for selfers is correct. Inbreeding depression trials are currently under way in this long-lived perennial (L. Rigney, unpublished data), and evidence to date shows that selfed ovules are more likely to abort than are outcrossed ovules, probably in part because of inbreeding depression. Selfed and outcrossed seeds germinate at equivalent rates, however, and the resulting plants grow at similar rates, as evidenced by annual exhumation and weighing of corms. Thus, inbreeding depression does occur in *E. grandiflorum,* but it is not very strong after seed formation, and it may not be of sufficient magnitude to explain the maintenance of an outcrossed mating system.

What about the presumed reproductive advantage of selfers? Are there reasons to think that it might not exist in spite of our calculations to the contrary? Yes, there are several. Our calculations for the reproductive advantage of selfers depend on the functional relationship between selfing and discounting rates embodied in the mass-action model we proposed. If that functional relationship is inappropriate for *E. grandiflorum,* we cannot use the relationship in equation (5) to infer the fate of variants with a higher selfing rate from our estimates of the success rates of self- and outcross pollen, because the derivation of equation (5) depends on the specific functional relationships assumed in that model, for example, in equation (4). One of the functional relationships implied by that model is clearly contradicted by our data. In developing our model we implicitly assumed that differences between individuals in the amount of self-pollen deposited are unrelated to differences in the amount of pollen removed from anthers. If this were true, success as an outcross pollen parent would decrease monotonically as the amount of self-pollen deposited increased, which would produce a negative correlation between self- and outcross pollen deposition. There is, as we noted above however, a statistically significant *positive* correlation between self- and outcross pollen deposition.

The functional relationship between selfing and discounting rates assumed in our model may be inappropriate for *E. grandiflorum* in another way. We assumed that the amount of pollen a plant is able to capture (π_i) is independent of the amount of pollen it deposits on its own stigma (δ_i). If self-deposition increases with the length of time a pollinator spends at each plant, as appears to be the case in *Erythronium* (J. D. Thomson, unpublished data), it is likely to be positively correlated with the receipt of outcross pollen, since long visits will allow the pollinator to deposit more grains (Thomson 1986). If so, the mechanics of pollen transfer may make it impossible to vary the number of pollen grains a plant deposits on its own stigmas without also varying the number of outcross pollen grains it receives on those stigmas. Under these conditions the rate of self-fertilization would be independent of the proportion of pollen devoted to selfing, because the rate of self-fertilization depends not only on the *amount* of self-pollen deposited but also on the *ratio* of self- to outcross pollen received. Reanalysis of the data collected in another study (Thomson and Stratton 1985) shows a nonsignificant positive correlation between the amount of pollen a plant deposits on its own stigmas and the amount of outcross pollen received on those stigmas (Pearson's $r = 0.30$, $N = 19$, $P > .05$). In that study, flowers had accumulated large amounts of nectar, and visits were very long. Under natural conditions, variation in visit length might produce an even stronger correlation.

In addition, this positive correlation between the amount of self-pollen deposited and the amount of outcross pollen received suggests that self-pollination in *E. grandiflorum* requires the visit of an insect pollinator; that is, it appears to be an instance of facilitated selfing (Lloyd and Schoen 1992). This is also consistent with the observation that caged plants rarely set fruit. As Lloyd (1992) has pointed out, facilitated selfing can provide some reproductive assurance when pollinators are limited (through increased seed set), but the extent of this assurance is reduced by the very factor that makes it necessary—pollinator limitation. Thus, facilitated selfing may not be strongly favored in *Erythronium,* even though insufficient pollination often limits fruit set (J. D. Thomson, unpublished data).

In short, it appears that pollination biology may have an important role to play in the maintenance of an outcrossed mating system in *E. grandiflorum,* but if it does, the role it plays is much different from what has been previously suggested (Holsinger 1988, 1991, 1992). There is no evidence for a trade-off between self-pollination and donation of outcross pollen to other plants. In fact, there is some evidence not only for a positive correlation between the amount of pollen deposited on self-stigmas and the amount of outcross pollen received on those same stigmas but also for a positive correlation between the amount of pollen a plant places on its own stigma and the amount of its pollen that reaches the stigmas of other plants. Outcrossing may be maintained in *E. grandiflorum* not because pollen discounting eliminates the transmission advantage of selfers or because inbreeding depression eliminates many selfed progeny but because the same processes that promote self-pollination—visits by pollinators—also promote the deposition of outcross pollen, which provides no mechanism by which the amount of self-pollen deposited can be increased without simultaneously increasing the amount of outcross pollen received. Pollen discounting does appear to play an important role in mating system evolution in *Mimulus* and *S. vulgaris,* but it cannot explain the maintenance of an outcrossed mating system in *E. grandiflorum*. It may also fail to explain the maintenance of an outcrossed mating system in other plants with similar patterns of pollen transfer.

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