

# Correlations among Fertility Components Can Maintain Mixed Mating in Plants

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**ABSTRACT:** Classical models studying the evolution of self-fertilization in plants conclude that only complete selfing and complete outcrossing are evolutionarily stable. In contrast with this prediction, 42% of seed-plant species are reported to have rates of self-ferti-

zation between 0.2 and 0.8. We propose that many previous models fail to predict intermediate selfing rates because they do not allow for functional relationships among three components of reproductive fitness: self-fertilized ovules, outcrossed ovules, and ovules sired by successful pollen export. Because the optimal design for fertility components may differ, conflicts among the alternative pathways to fitness are possible, and the greatest fertility may be achieved with some self-fertilization. Here we develop and analyze a model to predict optimal selfing rates that includes a range of possible relationships among the three components of reproductive fitness, as well as the effects of evolving inbreeding depression caused by deleterious mutations and of selection on total seed number. We demonstrate that intermediate selfing is optimal for a wide variety of relationships among fitness components and that inbreeding depression is not a good predictor of selfing-rate evolution. Functional relationships subsume the myriad effects of individual plant traits and thus offer a more general and simpler perspective on mating system evolution.

**Keywords:** functional relation, inbreeding depression, pollen discounting, self-fertilization, selective constraint, trade-off.

## Introduction

Cosexual organisms exhibit a wide range of rates of self-fertilization, from apparently complete selfing to partial selfing to complete outcrossing (Stebbins 1957; Schemske and Lande 1985; Goodwillie et al. 2005). The rate of self-fertilization is of special importance in evolution because it has both short-term and long-term fitness consequences, and these can act in opposition. Although individual se-

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lection determines whether self-fertilization will increase or decrease in the short term in a population (Lloyd 1992), the rate of self-fertilization may also directly affect several population-level properties, including the distribution and amount of genetic variation (Charlesworth and Charlesworth 1995; Ingvarsson 2002; Bakker et al. 2006), the probability of population extinction (Lynch et al. 1995), and the probability of giving rise to new clades (Stebbins 1957; Grant 1958; Wyatt 1988; Schoen et al. 1997; Takebayashi and Morrell 2001; Igic et al. 2004).

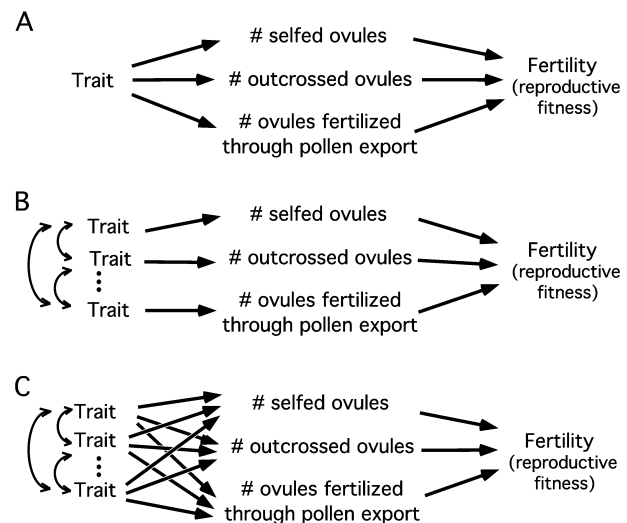
The evolution of rates of self-fertilization has been the subject of much experimental and theoretical research in recent years, especially in seed plants. A cosexual individual may achieve reproductive success in three ways: outcrossed ovules, selfed ovules, and ovules sired through pollen export. Compared with outcrossed ovules, self-fertilized ovules transmit twice as many genes, but this “automatic selection advantage” (Jain 1976) is reduced by inbreeding depression, the fitness reduction of selfed offspring relative to outcrossed offspring (Charlesworth and Charlesworth 1987). In addition, success through pollen export declines as the population level of self-fertilization increases. Models incorporating these three factors (automatic selection advantage, inbreeding depression, availability of outcrossed ovules) nearly always predict the evolution of either complete outcrossing or selfing (e.g., Lloyd 1979, 1992; Holsinger et al. 1984; Lande and Schemske 1985; Charlesworth et al. 1990; reviewed in Uyenoyama et al. 1993; Johnston 1998; Charlesworth 2006). In models with no other factors, inbreeding depression greater than one-half favors selfing and inbreeding depression less than one-half favors outcrossing.

The extent to which the basic models capture the important evolutionary forces shaping mating systems can be assessed by examining the distribution of selfing in nature. As predicted by the models, this distribution is bimodal, with more species clustered at the two extremes of 0%–20% and 81%–100% selfing (Schemske and Lande 1985; cf. Goodwillie et al. 2005). This bimodality strongly suggests that inbreeding depression is an important factor in the evolution of selfing rates. At the same time, despite the apparent bimodality, many species are partial selfers, with 42% having selfing rates between 20% and 80% (Goodwillie et al. 2005). The basic models cannot account for these species unless one assumes that these partial selfers are all slowly evolving to one extreme or the other.

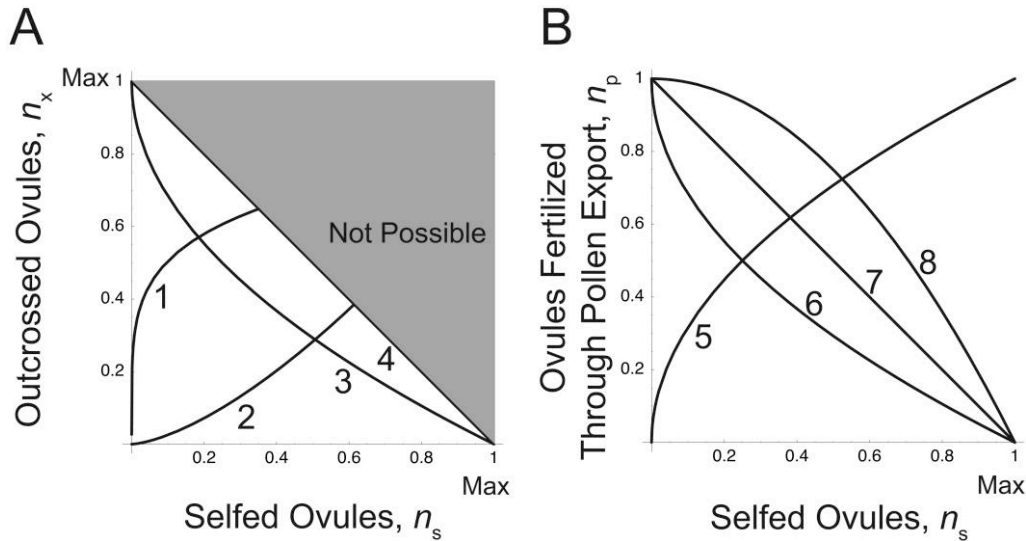
An alternative is that the basic models fail to capture one or more crucial aspects of the biology of reproduction and that the intermediate selfing rates are optimal because of stabilizing selection (Johnston 1998). The basic models treat the selfing rate—that is, the proportion of ovules that are self-fertilized rather than outcrossed—as the focal evolving character. This approach has two limitations.

First, it implicitly assumes a one-to-one genetic trade-off between the numbers of selfed and outcrossed ovules (complete “seed discounting”). There is, however, no reason to suppose that the available evolutionary options allow plants to trade one selfed ovule for one outcrossed ovule. In fact, a positive relationship between selfed and outcrossed ovules might often be expected, as for example when a larger floral display increases pollinator visitation and thus increases both selfed and outcrossed seed production. In addition to effects on maternal reproductive success, changing the number of selfed ovules might additionally affect success through pollen export in either negative (requiring “pollen discounting”) or positive ways (figs. 1, 2). Thus, it is expected that plants should exhibit a variety of functional relationships among the three fertility components and that these will constrain the evolution of the selfing rate. Some examples and biological bases for functional relationships are provided in table 1. A second limitation of the basic models is that they do not allow for the fact that selection on other fitness components, such as allocation to total seed number, can set limits on the numbers of selfed and outcrossed ovules (fig. 2, *gray region*; see also Lloyd 1979, p. 73).

In this article, we employ the methods of Johnston (1998) to model the evolution of the selfing rate under any set of functional relationships among number of selfed ovules, number of outcrossed ovules, and pollen export



**Figure 1:** Origin of functional relationships among the three fertility components in cosexual organisms. Functional relationships among fertility components will arise when an underlying trait influences more than one component (A). They will also arise when each trait directly affects only one fertility component but the traits are correlated (B). While either of these causes is sufficient, real biological systems will generally exhibit both (C).



**Figure 2:** Two functional relationships among the three fertility components, with example functions. *A*, Number of outcrossed ovules as a function of number of selfed ovules. *B*, Pollen export success as a function of number of selfed ovules. “Max” refers to the maximum number of ovules that can mature to seeds. Functions may not enter the gray region, because there the maximum number of seeds is exceeded. Fertilized ovules and successful pollen numbers expressed as a fraction of total maximum seed number are indicated by  $n_x$ ,  $n_s$ , and  $n_p$ . In example functions 1 and 2, some selfing occurs as a result of outcrossing (and vice versa), as might occur with geitonogamy. Functions 3 and 4 represent a trade-off between selfed and outcrossed ovules (seed discounting); function 4 is the classical 1 : 1 trade-off. Plants characterized by function 5 have increased selfing with increased pollen export success, whereas functions 6–8 represent decreased pollen export success with increasing selfing (requires pollen discounting). Functions representing Lloyd’s (1979, 1992) modes of selfing are also readily accommodated.

success. We allow inbreeding depression to evolve with the population average selfing rate. We find that functional relationships among fertility components, in combination with selection on maximum seed number, often result in stable (locally optimal) intermediate selfing rates. Focusing on functional relationships among fertility components, instead of on the selfing rate per se, will improve our understanding of the evolution of mating systems. Because functional relationships subsume the myriad effects of numerous plant traits, they offer a more general and simpler perspective on mating system evolution.

#### Rationale for Model Construction

In a particular population, the total number of fertilized ovules and the pollen export success might each be related to the selfing rate, as discussed above. Because we are interested in evolution of the selfing rate itself, it might initially appear to be most reasonable to model fitness as a function of total number of fertilized ovules and of pollen export success, each of which is some particular function of the selfing rate. Under this construction, the total number of fertilized ovules is made a function of selfing rate, which itself contains as its denominator the total number of fertilized ovules (i.e.,  $N_{\text{total}} = \text{function}(N_s/N_{\text{total}}) =$

$\text{function}(N_s/(N_s + N_x))$ , where  $N_{\text{total}}$  is the total number of fertilized ovules and  $N_s$  and  $N_x$  are the numbers of selfed and outcrossed ovules). A complete investigation of selfing rate evolution should include all biologically possible relationships between numbers of selfed and outcrossed ovules. Unfortunately, for many functions relating total numbers of fertilized ovules to the selfing rate, there will not exist a functional relationship between the numbers of selfed and outcrossed ovules. As a result, investigating the variety of functions relating  $N_{\text{total}}$  to the selfing rate  $S$  would both exclude possible relationships between  $N_x$  and  $N_s$  and include unwanted, nonfunctional relationships. Therefore, in order to study all possible relationships between numbers of selfed and outcrossed ovules  $N_x$  and  $N_s$ , it is preferable to establish relationships between these two quantities and then investigate  $S$  as a function of them, rather than to model the total number of fertilized ovules as a function of the selfing rate directly.

#### General Model

We consider the absolute fitness  $w_{\text{mutant}}$  of an individual with a phenotype differing from that of the other members of the population. This individual produces a number of self-fertilized ovules  $N_s$  and a number of outcrossed ovules

**Table 1:** Biological bases of the functional relationships among the three fertility components: number of selfed ovules, number of outcrossed ovules, and success through pollen export

Increase in no. selfed ovules ( $n_s$ ) is associated with:	Biological basis (references)
No. outcrossed ovules ( $n_x$ )	
Increased $n_x$	Increased attractiveness (e.g., flower size, nectar quantity, or display size) causes more pollinator visits but also increases transfer of self-pollination among flowers (geitonogamy; e.g., Karron et al. 2004; Williams 2007) or within flowers (facilitated selfing; e.g., van Kleunen and Ritland 2004).
Decreased $n_x$	Without pollen limitation, prior or competing selfing uses ovules that would otherwise be outcrossed (ovule or seed discounting; e.g., Herlihy and Eckert 2002).
Pollen export success ( $n_p$ )	
Increased $n_p$	Higher pollinator visitation rates increase geitonogamous selfing but also rate of pollen export (e.g., Harder and Thomson 1989; Jersakova and Johnson 2007). Longer stamens (hence, smaller stigma-anther separation in long-styled flowers) increase selfing but may also contact pollinators more efficiently (e.g., Kohn and Barrett 1994).
Decreased $n_p$	Pollen retained for selfing is not exported, resulting in decreased outcross pollen success. Pollen discounting; documented numerous times (e.g., Rademaker and DeJong 1998; Harder et al. 2000; Fishman 2000; see Harder and Wilson 1998 for discussion of conceptions and effects of pollen discounting).

$N_x$ . Because we are not studying the evolution of seed number, we assume that the focal individual and all other individuals have a maximum number of ovules that can be fertilized  $N_{\max}$ , and we express the numbers of actually fertilized ovules  $n$  as proportions of this maximum. Thus,  $n_s = N_s/N_{\max}$  is the proportion of selfed ovules among all fertilizable ovules (i.e., the probability that an ovule is self-fertilized) and  $n_x = N_x/N_{\max}$  is the proportion of outcrossed ovules among all fertilizable ovules (i.e., the probability that an ovule is outcrossed). Whenever all fertilizable ovules are fertilized ( $n_s + n_x = 1$ ),  $n_s$  is the selfing rate  $S$  and  $n_x$  is the outcrossing rate  $1 - S$ . Next, we establish functions relating selfed ovules, outcrossed ovules, and pollen export success to one another (the functional relationships); it is immaterial which of these quantities is chosen as the independent variable. Here we define outcrossed ovules and pollen export proficiency as functions  $f_x$  and  $f_p$  of selfed ovule production (fig. 2). For the rare phenotype, reproductive success achieved through pollen export depends on its pollen export proficiency  $f_p(n_s)$  relative to that of the other members of the population  $f_p(\bar{n}_s)$  and on the average number of outcrossed ovules produced by the common phenotype  $f_x(\bar{n}_s)$ . Fitness of the mutant phenotype can be represented as

$$w_{\text{mutant}} = 2(1 - \delta(\bar{n}_s))n_s + f_x(n_s) + \frac{f_p(n_s)}{f_p(\bar{n}_s)}f_x(\bar{n}_s), \quad (1a)$$

where the population inbreeding depression  $\delta(\bar{n}_s)$  evolves with the mean selfing rate (described below). Selection on the number of selfed ovules, indicated by the partial de-

rivative of fitness with respect to number of selfed ovules, is

$$\frac{\partial w_{\text{mutant}}}{\partial n_s} = 2(1 - \delta(\bar{n}_s)) + f'_x(n_s) + f'_p(n_s)\frac{1}{f_p(\bar{n}_s)}f_x(\bar{n}_s). \quad (1b)$$

This partial derivative gives the change in fitness with change in number of selfed ovules for any particular inbreeding depression and number of outcrossed ovules of the common phenotype. It shows that total selection on the number of selfed ovules depends not only on the direct relationship between selfed ovules and fitness (slope =  $2(1 - \delta)$ ), but also on correlational selection acting on number of outcrossed ovules and pollen export success. The quantity  $2(1 - \delta(\bar{n}_s))$  represents the fertility contribution from self-fertilized ovules, the quantity  $f'_x(n_s)$  is the fertility contribution from outcrossed ovules, and the product  $f'_p(n_s)(1/f_p(\bar{n}_s))f_x(\bar{n}_s)$  is the fertility contribution from pollen export. The quantity  $1/f_p(\bar{n}_s)$  is the reciprocal of the pollen export fertility of the common phenotype and is always positive. The quantity  $f_x(\bar{n}_s)$  is the number of outcrossed ovules of the common phenotype and can take values from 0 to 1, inclusive. The product of quantities  $1/f_p(\bar{n}_s)$  and  $f_x(\bar{n}_s)$  is therefore positive or zero.

The curvature of the selection surface, in the plane of fitness versus selfed ovules, is the second partial derivative with respect to number of selfed ovules:

$$\frac{\partial^2 w_{\text{mutant}}}{\partial n_s^2} = f''_x(n_s) + f''_p(n_s)\frac{1}{f_p(\bar{n}_s)}f_x(\bar{n}_s). \quad (1c)$$

The standard condition for  $\hat{n}_s$  to be an evolutionarily stable strategy (ESS) is that, when all individuals adopt the same strategy, the selection surface in the plane of the character of interest has a maximum at some point within the range of the character of interest, which in this case is the number of selfed ovules. Any individual deviating from the common phenotype suffers a fitness loss. In short,

$$\left. \frac{\partial(w_{\text{mutant}})}{\partial n_s} \right|_{n_s = \hat{n}_s} = 0,$$

$$\left. \frac{\partial^2(w_{\text{mutant}})}{\partial n_s^2} \right|_{n_s = \hat{n}_s} < 0.$$

These conditions are valid when fitness is an increasing or decreasing monotone function over the range of the number of selfed ovules. Because we have imposed the constraint that there is some maximum number of ovules that are fertilized, most relationships between numbers of outcrossed and selfed ovules other than an exact trade-off will in fact not extend over the range of complete outcrossing to complete selfing (fig. 2A). Therefore, further evolutionary change will often be halted, and stable intermediate selfing rates result from the functional relationships in combination with selection on total seed number.

Our approach assumes that the selfing rate evolves most readily, inbreeding depression evolves as a consequence, and the functional relationships are fixed. Although it is clear that functional relationships can evolve, it seems reasonable to assume that they generally evolve more slowly than the selfing rate. The present theory also assumes that the mating system evolves independently of the loci determining inbreeding depression. Theory suggests that genetic associations (identity disequilibria) between mating system modifiers and fitness loci can qualitatively alter predictions (Holsinger 1988; Uyenoyama and Waller 1991). If allelic variation in the selfing rate is slight, however, these associations should be minor.

### Evolution of Inbreeding Depression

To obtain equilibrium inbreeding depression as a function of the mean selfing rate  $\delta(\bar{S})$ , we first implemented computer simulations of a modified version of Kondrashov's (1985) model, which gives the distribution of number of deleterious mutations per individual in an infinite population under a given selfing rate. This model assumes a very large (effectively infinite) number of unlinked loci mutating at genomic rate  $U$  to deleterious alleles with dominance coefficient  $h$  and selection coefficient  $s$ ; each

mutation occurs at a new locus (or one not currently segregating in the population) and is therefore unique. The inbreeding depression due to deleterious mutations is determined by the distribution of numbers of heterozygous and homozygous mutations per individual (e.g., Charlesworth et al. 1990). Under a given combination of parameter values (mutation rate, dominance, selection coefficient), we ran separate simulations for selfing rates ranging from 0 to 1 in steps of 0.01 (fig. 3). As in Johnston (1998), these values were then fitted by polynomial regression of degree 5 ( $R^2 \gg 0.99$ ), yielding the formulas relating equilibrium inbreeding depression to population selfing rate that are used in our model.

### Model Results

Whenever numbers of selfed and outcrossed ovules have an intrinsic positive genetic correlation, the ESS selfing rate is intermediate (fig. 4). A positive relationship between numbers of selfed ovules and numbers of outcrossed ovules means that selfing entails outcrossing, and vice versa. When selfing does not diminish pollen export success, the ESS selfing rate is determined jointly by the functional relationship between selfed and outcrossed ovules and maximum seed number (fig. 4A). At some point, a further increase in selfed ovule number is halted by selection on maximum seed number. Thus, in figure 4A, the ESS selfing rate is 0.35 under all mutation models because selection favors higher selfed ovule numbers everywhere below the maximum, and this is the proportion of selfed ovules at maximum seed number. Note that positive relationships among fertility components will cause the evo-

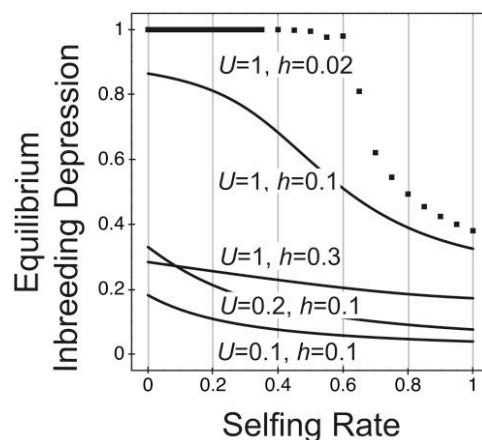
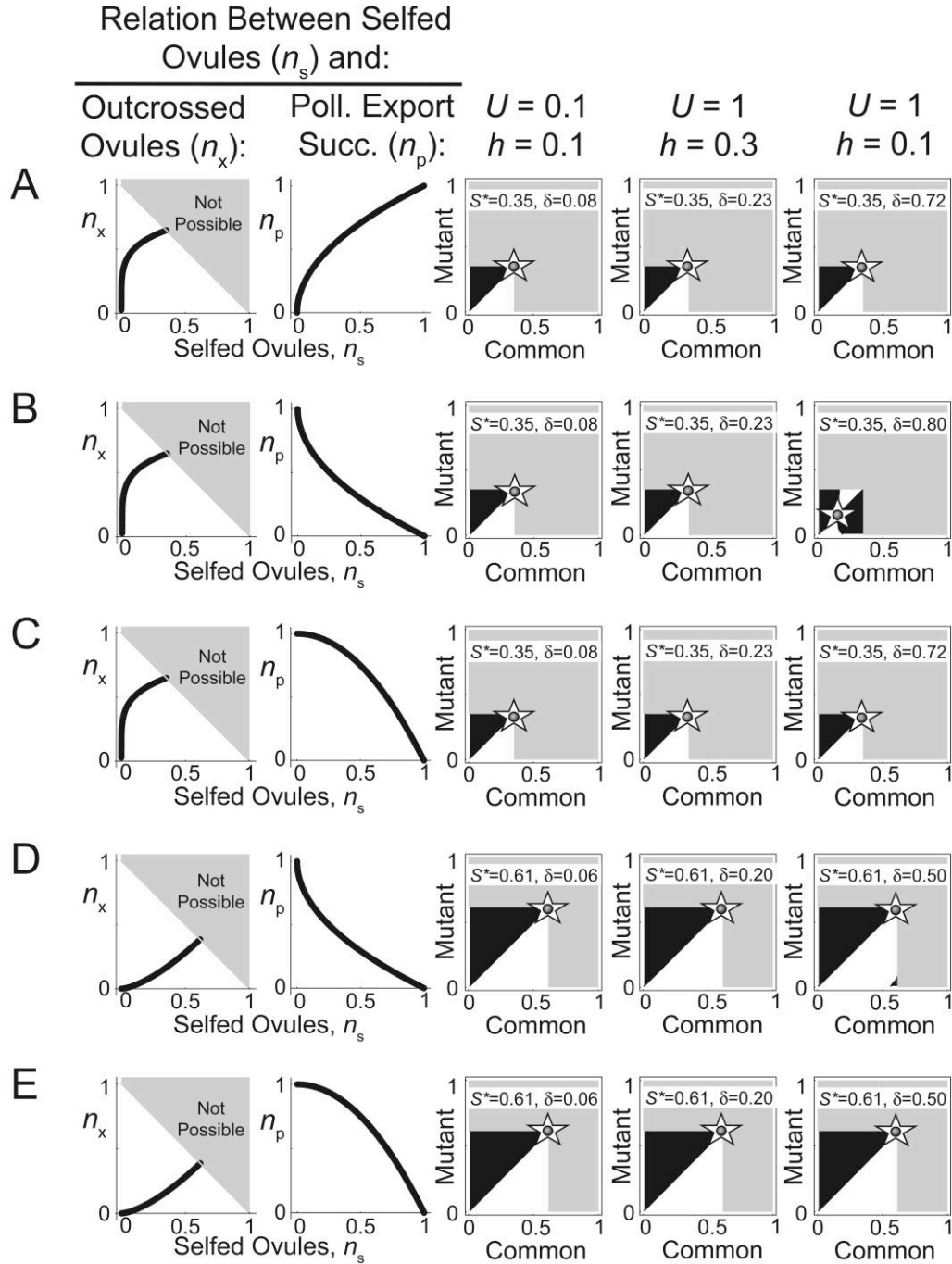


Figure 3: Relationship between equilibrium inbreeding depression and population selfing rate for several values of mutation rate ( $U$ ) and dominance coefficient ( $h$ ). Selection coefficient against mutations is 0.2 in all cases.



**Figure 4:** Evolutionarily stable strategy (ESS) selfing rates and inbreeding depression with positive functional relationships between selfed and outcrossed ovules. Functional relationships depict number of self-fertilized ovules versus number of outcrossed ovules (leftmost graph in each row) and versus success from pollen export (second graph in each row). All relationships in this figure represent negative seed discounting; *B–E* represent pollen discounting. The resulting fitness surfaces under three mutation models are represented as contour plots (pairwise invasibility plots), where black areas represent higher fitness of the rare mutant compared with the common form and white areas represent lower fitness of the mutant; gray areas are not available because of selection on total seed number. The evolutionarily stable rate of self-fertilization ( $S^*$ ) is represented by a star on each plot, and the associated equilibrium inbreeding depression is given as  $\delta$ . Functional relationships are  $n_s = b_x + (m_x - b_x)n_x^c$  and  $n_p = b_p + (m_p - b_p)n_p^c$ , where  $b_x$ ,  $m_x$ ,  $c_x$ ,  $b_p$ ,  $m_p$ , and  $c_p$  are as follows: 0, 0.8, 0.2, 0, 1, 0.5 (A); 0, 0.8, 0.2, 1, 0, 0.5 (B); 0, 0.8, 0.2, 1, 0, 2 (C); 0, 0.8, 1.5, 1, 0, 0.5 (D); and 0, 0.8, 1.5, 1, 0, 2 (E), respectively. For the functional relationships depicted here, ESS selfing rates under mutation model  $U = 0.2$  and  $h = 0.1$  are the same as for  $U = 0.1$  and  $h = 0.1$ , with higher equilibrium inbreeding depression.

lution of increased selfed ovule number even when inbreeding depression greatly exceeds one-half (e.g., fig. 4A;  $U = 1$ ,  $h = 0.1$ , equilibrium inbreeding depression ( $\hat{\delta}$ ) = 0.72).

When selfed and outcrossed ovule numbers increase together but there is pollen discounting, the ESS selfing rate is again intermediate (fig. 4B–4E). In these conditions, the further increase of selfed ovules can be halted if pollen discounting is sufficiently severe. For example, in figure 4B,  $U = 1$ ,  $h = 0.1$ , the ESS selfing rate is 0.22, and the total number of mature ovules is less than the maximum. In all other examples of pollen discounting with a positive relationship between selfed and outcrossed ovules (fig. 4B–4E), the ESS selfing rate is determined by the maximum seed number.

In contrast with the case of a positive relationship between selfed and outcrossed ovules, stable selfing rates of 0 and 1 can arise when there is a trade-off between selfed and outcrossed ovules (fig. 5). In these cases, if pollen export success increases with number of selfed ovules, then the optimal selfing rate is 1, regardless of the mutation model (fig. 5A). In contrast, with pollen discounting, the optimal selfing rate can be 0, intermediate, or 1 (fig. 5B–5E). When increasing the number of selfed ovules decreases total number of fertilized ovules, then there is disruptive selection and the optimal selfing rate is 0 or 1 (fig. 5B). When there is an exact trade-off between selfed and outcrossed ovules (fig. 5C–5E), the ESS selfing rate depends on pollen discounting and the mutation model. When pollen discounting is complete (fig. 5C) or becomes less severe with an increased selfed-ovule number (fig. 5D), then complete selfing is selected. When, on the other hand, pollen discounting becomes more severe with increasing numbers of selfed ovules, intermediate selfing rates will often arise (fig. 5E).

### Discussion

In this article, we explored a model of optimal selfing rates that included a range of possible relationships among the three components of reproductive fitness, as well as the effects of evolving inbreeding depression caused by deleterious mutations. This approach is general: although individual traits influence the three fertility components in specific, often interacting ways, this very large number of relationships can all be condensed to three functional relationships among fertility components. Functional relationships offer a different perspective from one given by studying particular factors such as mode of selfing, reproductive assurance, reproductive compensation, pollen discounting, and ovule or seed discounting (for discussion and results, see Lloyd 1992; Harder and Wilson 1998; Johnston 1998; Cheptou 2004; Goodwillie et al. 2005; Porcher

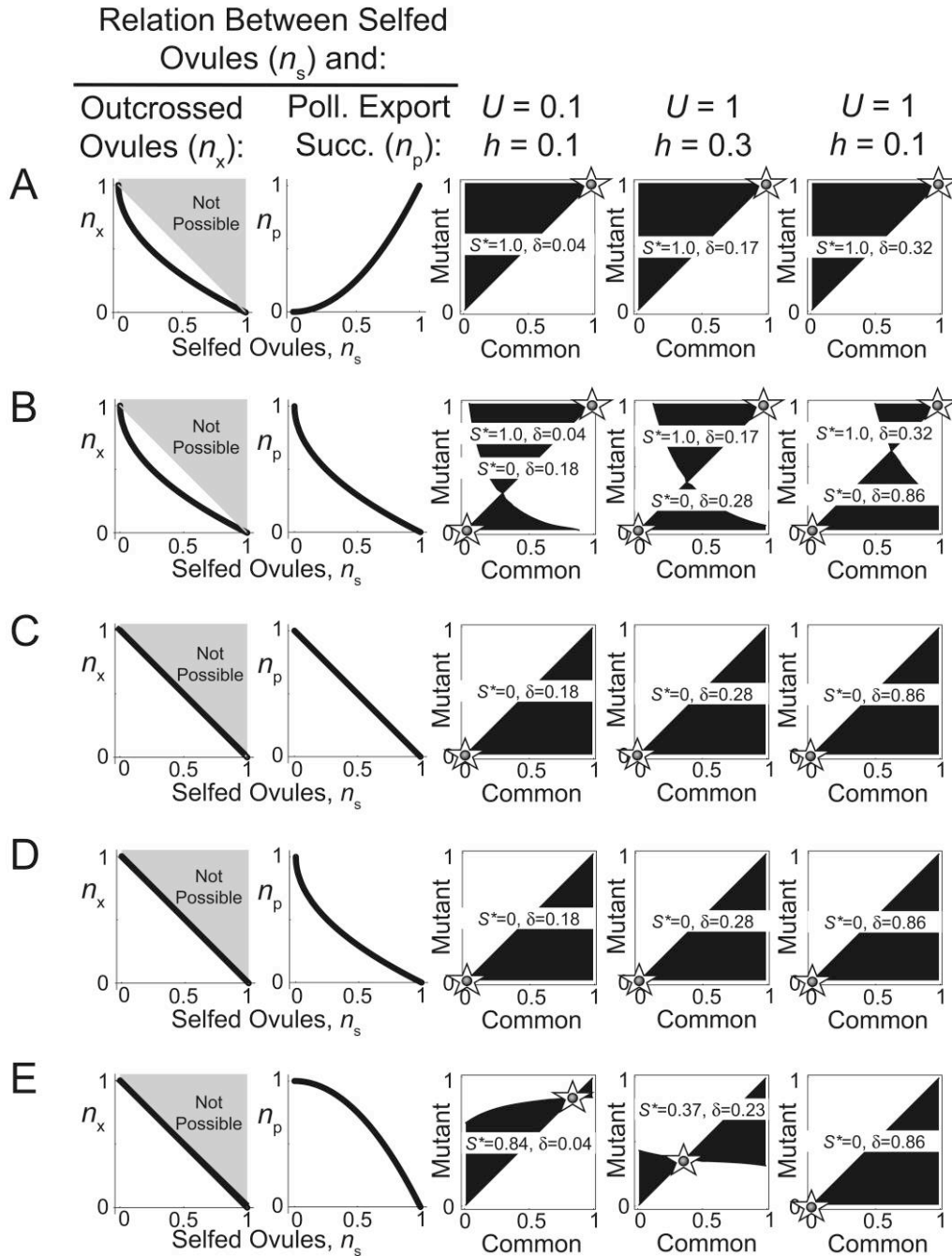
and Lande 2005a, 2005b; Harder et al. 2008 and references within). In general, a particular functional relationship will result from a number of such factors.

The models developed here showed that intermediate selfing is optimal for a wide variety of relationships among fitness components that have been found or expected in natural populations. In fact, the models suggest that intermediate selfing should be quite common. Goodwillie et al. (2005) found that 42% of 345 species had selfing rates between 0.2 and 0.8. The proportion of mixed mating is still larger when one considers that the ranges 0.0–0.2 and 0.8–1.0 hide much mixed mating. Unfortunately, the data set is not a random sample; it is biased by the species chosen for the study, 44% of which belong to only five families (Goodwillie et al. 2005). Whether further estimates cause the proportion to increase or decrease, it is clear that many species engage in mixed mating.

### Testing the Models

Our models show that the selfing rate should often experience stabilizing selection. This is in contrast with the view that the selfing rate experiences only directional selection, either positive or negative in different populations, and that intermediate rates are transient. The best way to distinguish between these opposing hypotheses is to measure linear and quadratic selection on selfing. This is challenging because of uncertainties in the estimation of several quantities, including male outcrossing success, individual selfing rates, inbreeding depression, and total fitness. In addition, stabilizing selection coefficients are notoriously recalcitrant to statistical significance (Kingsolver et al. 2001; Blows and Brooks 2003).

Our models predict that stable intermediate selfing rates will occur in two general situations. The first is when there is a positive functional relationship between numbers of selfed and outcrossed ovules. This can be tested by assessing the sign of the genetic correlation between numbers of selfed and outcrossed ovules under natural conditions. Second, mixed mating will often also occur with a negative relationship (trade-off) between selfed and outcrossed ovules when pollen discounting becomes more severe with increased numbers of selfed ovules (Johnston 1998). Again, the functional relationship can be tested in natural conditions, but here a simple negative correlation is not sufficient to determine the increasing severity of pollen discounting. The model considered here assumes that all traits other than inbreeding depression, maximum seed number, and the fertility functional relationships can be considered to be constant. In testing the model, it will thus be important to control as much as possible for variation in other plant features, such as total size. Genetic correlations can be measured either by using standard breeding



**Figure 5:** Evolutionarily stable strategy (ESS) selfing rates and inbreeding depression with negative functional relationships between selfed and outcrossed ovules. All relationships in this figure represent seed discounting; *B–E* represent pollen discounting. See the legend for figure 4. Parameter values for  $b_s$ ,  $m_s$ ,  $c_s$ ,  $b_p$ ,  $m_p$ , and  $c_p$  in the two functional relationships are 1, 0, 0.5, 0, 1, 2 (*A*); 1, 0, 0.5, 1, 0, 0.5 (*B*); 1, 0, 1, 1, 0, 1 (*C*); 1, 0, 1, 1, 0, 0.5 (*D*); and 1, 0, 1, 1, 0, 2 (*E*), respectively. ESS selfing rates under mutation model  $U = 0.2$  and  $h = 0$  are the same as for  $U = 0.1$  and  $h = 0.1$  in *A–D*, but with higher equilibrium inbreeding depression; for *E*, ESS selfing rate is 0.66 and equilibrium inbreeding depression is 0.10.



designs (Lynch and Walsh 1998) or response to artificial selection on one of the three fertility components. Artificial selection could be performed, for example, on the number of selfed ovules under natural conditions. The response to selection on the other two fertility components would reveal the functional relationships.

### Functional Relationships as Constraints

The functional relationships considered in this article act as constraints. Selective and developmental constraints have been actively discussed, and distinguishing between them is often difficult (e.g., Maynard Smith et al. 1985; Arnold 1992; Perrin and Travis 1992; Ghalambor et al. 2004). Developmental constraints are those that influence which phenotypes can be produced, whereas selective constraints arise from the requirement that natural selection produce fitter phenotypes. The functional relationships considered here define the ways that genetically based changes to one fertility component affect another component. As a result, given a particular set of relationships, there will be some optimal level of self-fertilization. In this sense, the relationships among fertility components clearly act as selective constraints because, when at a local optimum, any phenotypic change causes a decrease in total fertility. The very existence of the functional relationships, however, means that populations can evolve only along them. In this sense, the functional relationships act more like developmental constraints because they are considered to be constant. An important unresolved question, then, is to what degree these functional relationships can themselves evolve. It is clear that they can, because there is an evolutionary pathway connecting the great variety of functional relationships displayed by species. Nevertheless, it is likely that, once established, the general shape of these relationships guides the evolutionary process and thus acts as a constraint.

### Total Seed Number as a Hard Constraint

Optimal selfing rates are clearly not only affected by inbreeding depression but also by the functional relationships among the three components of fertility. Furthermore, other traits, such as allocation, will have important influences on the ESS selfing rate. This is most clearly illustrated in the present analysis by selection on total seed number. For example, in figure 4, numbers of outcrossed and selfed ovules increase together, and higher numbers of selfed ovules are often favored. Complete selfing does not evolve, however, because of the functional relationship between selfed and outcrossed ovules: selfing entails some outcrossing (and vice versa), and increasing the number of selfed ovules can only continue until maximum total

seed number is reached. This maximum seed number is set by factors outside of those affecting numbers of selfed and outcrossed seeds. In this example, total seed number constrains evolution of the selfing rate. Note that high inbreeding depression can establish stable intermediate selfing rates that are below the maximum seed number (fig. 4B;  $U = 1$ ,  $h = 0.1$ ).

We have assumed that seed number does not evolve with the mating system, and therefore we assumed a maximum number of ovules that can mature to seeds. Whenever numbers of selfed and outcrossed ovules are positively genetically correlated, the ESS selfing rate will generally be intermediate. A concern might therefore be raised that stable intermediate selfing is somehow built into the model. There are three important answers to this potential concern. First, our model is general, allowing any relationship between fertility components. The one-to-one trade-off of the basic models represents a specific case. As shown previously (Johnston 1998), we find that stable intermediate selfing rates can arise in this situation when pollen discounting becomes stronger in a nonlinear fashion with increased selfing (e.g., fig. 5E). Second, correlations among fertility components are exactly the point: for some kinds of floral structure and some types of pollinator there will exist a particular functional relationship between numbers of selfed and outcrossed ovules. That is, the available evolutionary options do not permit trading off selfed and outcrossed ovules so that complete selfing or outcrossing can be achieved. Third, one could remove the absolute constraint of a maximum number of fertilizable ovules by including seed number as a trait under selection. In general, the effect would be a slight shift in the optimal selfing rate, the amount of which depends on the relative strengths of selection on total seed number and the other fertility components.

### Is Partial Selfing “Adaptive”?

The main idea presented here is that the limited evolutionary options for changing the selfing rate also affect other fertility components. In such a situation, total selection on the selfing rate can be stabilizing; thus, partial selfing is adaptive and evolutionarily stable. The idea that plants have limited evolutionary options for adjusting the selfing rate is captured well by Holsinger and Thomson (1994, p. 810) in their study of *Erythronium grandiflorum* (Liliaceae): “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.” The converse—that some selfing occurs as a necessary result of outcrossing—will probably be true even more often. A broad view that takes into account all aspects of fertility, or indeed total fitness, will often cause us to regard intermediate selfing rates as adaptive: given the evolutionary options (described by the functional relationships), the highest fitness results when some selfing occurs. Alternatively, if we focus only on inbreeding depression, the automatic selection advantage, or a subset of the fertility components, then we will regard partial selfing as a nonadaptive consequence of selection on other fitness components. For example, “the selfing component to mixed mating might arise through geitonogamy and might be a nonadaptive cost that is associated with the large floral displays that are typically required to attract animal pollinators” (Barrett 2002, p. 282; see also Eckert 2000). We advocate the broader view because it uses total fitness to define adaptation.

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#### Literature Cited

- Arnold, S. J. 1992. Constraints on phenotypic evolution. *American Naturalist* 140(suppl.):S85–S107.
- Bakker, E. G., E. A. Stahl, C. Toomajian, M. Nordborg, M. Kreitman, and J. Bergelson. 2006. Distribution of genetic variation within and among local populations of *Arabidopsis thaliana* over its species range. *Molecular Ecology* 15:1405–1418.
- Barrett, S. C. H. 2002. The evolution of plant sexual diversity. *Nature Reviews Genetics* 3:274–284.
- Blows, M. W., and R. Brooks. 2003. Measuring nonlinear selection. *American Naturalist* 162:815–820.
- Charlesworth, D. 2006. Evolution of plant breeding systems. *Current Biology* 16:R726–R735.
- Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18:237–268.
- . 1995. Quantitative genetics in plants: the effect of the breeding system on genetic variability. *Evolution* 49:911–920.
- Charlesworth, D., M. T. Morgan, and B. Charlesworth. 1990. Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multilocus system with no linkage. *Evolution* 44:1469–1489.
- Cheptou, P.-O. 2004. Allee effect and self-fertilization in hermaphrodites: reproductive assurance in demographically stable populations. *Evolution* 58:2613–2621.
- Eckert, C. G. 2000. Contributions of autogamy and geitonogamy to self-fertilization in a mass-flowering, clonal plant. *Ecology* 81:532–542.
- Fishman, L. 2000. Pollen discounting and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). *Evolution* 54:1558–1565.
- Ghalambor, C. K., D. N. Reznick, and J. A. Walker. 2004. Constraints on adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). *American Naturalist* 164:38–50.
- Goodwillie, C., S. Kalisz, and C. G. Eckert. 2005. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology, Evolution and Systematics* 36:47–79.
- Grant, V. 1958. The regulation of recombination in plants. *Cold Spring Harbor Symposia on Quantitative Biology* 23:337–363.
- Harder, L. D., and J. D. Thomson. 1989. Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. *American Naturalist* 133:323–344.
- Harder, L. D., and W. G. Wilson. 1998. A clarification of pollen discounting and its joint effects with inbreeding depression on mating system evolution. *American Naturalist* 152:684–695.
- Harder, L. D., S. C. H. Barrett, and W. W. Cole. 2000. The mating consequences of sexual segregation within inflorescences of flowering plants. *Proceedings of the Royal Society B: Biological Sciences* 267:315–320.
- Harder, L. D., S. A. Richards, and M. B. Routley. 2008. Effects of reproductive compensation, gamete discounting and reproductive assurance on mating-system diversity in hermaphrodites. *Evolution* 62:157–172.
- Herlihy, C. R., and C. G. Eckert. 2002. Genetic cost of reproductive assurance in a self-fertilizing plant. *Nature* 416:320–323.
- Holsinger, K. E. 1988. Inbreeding depression doesn’t matter: the genetic basis of mating system evolution. *Evolution* 42:1235–1244.
- Holsinger, K. E., and J. D. Thomson. 1994. Pollen discounting in *Erythronium grandiflorum*: mass-action estimates from pollen transfer dynamics. *American Naturalist* 144:799–812.
- Holsinger, K. E., M. W. Feldman, and F. B. Christiansen. 1984. The evolution of self-fertilization in plants: a population genetic model. *American Naturalist* 124:446–453.
- Igic, B., L. Bohs, and J. R. Kohn. 2004. Historical inferences from the self-incompatibility locus. *New Phytologist* 161:97–105.
- Ingvarsson, P. K. 2002. A metapopulation perspective on genetic diversity and differentiation in partially self-fertilizing plants. *Evolution* 56:2368–2373.
- Jain, S. K. 1976. The evolution of inbreeding in plants. *Annual Review of Ecology and Systematics* 7:469–495.
- Jersakova, J., and S. D. Johnson. 2007. Protandry promotes male pollination success in a moth-pollinated orchid. *Functional Ecology* 21:496–504.

- Johnston, M. O. 1998. Evolution of intermediate selfing rates in plants: pollination ecology versus deleterious mutations. *Genetica* 103:267–278.
- Karron, J. D., R. J. Mitchell, K. G. Holmquist, J. M. Bell, and B. Funk. 2004. The influence of floral display size on selfing rates in *Mimulus ringens*. *Heredity* 92:242–248.
- Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. *American Naturalist* 157:245–261.
- Kohn, J. R., and S. C. H. Barrett. 1994. Pollen discounting and the spread of a selfing variant in tristylous *Eichhornia paniculata*: evidence from experimental populations. *Evolution* 48:1576–1594.
- Kondrashov, A. S. 1985. Deleterious mutations as an evolutionary factor. II. Facultative apomixis and selfing. *Genetics* 111:635–653.
- Lande, R., and D. W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* 39:24–40.
- Lloyd, D. G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. *American Naturalist* 113:67–97.
- . 1992. Self-fertilization and cross-fertilization in plants. II. The selection of self-fertilization. *International Journal of Plant Sciences* 153:370–380.
- Lynch, M., and B. Walsh. 1998. *Genetics and analysis of quantitative traits*. Sinauer, Sunderland, MA.
- Lynch, M., J. Conery, and R. Burger. 1995. Mutation accumulation and the extinction of small populations. *American Naturalist* 146: 489–518.
- Maynard Smith, J., R. Burian, S. Kauffman, P. Alberch, J. Campbell, B. Goodwin, R. Lande, D. Raup, and L. Wolpert. 1985. Developmental constraints and evolution: a perspective from the Mountain Lake Conference on Development and Evolution. *Quarterly Review of Biology* 60:265–287.
- Perrin, N., and J. Travis. 1992. On the use of constraints in evolutionary biology and some allergic reactions to them. *Functional Ecology* 6:361–363.
- Porcher, E., and R. Lande. 2005a. The evolution of self-fertilization and inbreeding depression under pollen discounting and pollen limitation. *Journal of Evolutionary Biology* 18:497–508.
- . 2005b. Reproductive compensation in the evolution of plant mating systems. *New Phytologist* 166:673–684.
- Rademaker, M. C. J., and T. J. De Jong. 1998. Effects of flower number on estimated pollen transfer in natural populations of three hermaphroditic species: an experiment with fluorescent dye. *Journal of Evolutionary Biology* 11:623–641.
- Schemske, D. W., and R. Lande. 1985. The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution* 39:41–52.
- Schoen, D. J., M. O. Johnston, A.-M. L'Heureux, and J. V. Marsolais. 1997. Evolutionary history of the mating system in *Amsinckia* (Boraginaceae). *Evolution* 51:1090–1099.
- Stebbins, G. L. 1957. Self-fertilization and population variability in the higher plants. *American Naturalist* 91:337–354.
- Takebayashi, N., and P. Morrell. 2001. Is self-fertilization a dead end? revisiting an old hypothesis with genetic theories and a macro-evolutionary approach. *American Journal of Botany* 88:1143–1150.
- Uyenoyama, M. K., and D. M. Waller. 1991. Coevolution of self-fertilization and inbreeding depression. I. Mutation-selection balance at one and two loci. *Theoretical Population Biology* 40:14–46.
- Uyenoyama, M. K., K. E. Holsinger, and D. M. Waller. 1993. Ecological and genetic factors directing the evolution of self-fertilization. *Oxford Surveys in Evolutionary Biology* 9:327–381.
- van Kleunen, M., and K. Ritland. 2004. Predicting evolution of floral traits associated with mating system in a natural plant population. *Journal of Evolutionary Biology* 17:1389–1399.
- Williams, C. F. 2007. Effects of floral display size and biparental inbreeding on outcrossing rates in *Delphinium barbeyi* (Ranunculaceae). *American Journal of Botany* 94:1696–1705.
- Wyatt, R. 1988. Phylogenetic aspects of the evolution of self-pollination. Pages 109–131 in L. D. Gottlieb and S. K. Jain, eds. *Plant evolutionary biology*. Chapman & Hall, London.

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Left, Selection for traits such as clonal reproduction can favor stable intermediate levels of self-fertilization. Shown here: *Iris versicolor* in Nova Scotia. Right, Partial self-fertilization is expected to be optimal in many species because flowers serve several functions, and the selfing rate cannot be adjusted independently of them. Shown here: *Rhododendron canadense* in Nova Scotia. Photographs by M. O. Johnston.