

Evolution of intermediate selfing rates in plants: pollination ecology versus deleterious mutations

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Key words: dominance, inbreeding depression, mutation rate, pollination, self-fertilization

Abstract

The evolutionarily stable rate of self-fertilization is studied in phenotypic models that incorporate pollination ecology as well as the correlated evolution of inbreeding depression and the population mean selfing rate. Inbreeding depression is assumed to be caused by continual mutation to deleterious, partially recessive alleles. Several mutation rates and dominance levels are included. Two separate ecological cases are studied: how selfing rate affects proportion of ovules fertilized (pollination assurance, seed discounting) and how selfing rate affects male outcrossing success through pollen discounting. Evolutionarily stable rates are invariably zero or intermediate in two circumstances, namely when increased selfing causes (1) a decrease in the proportion of ovules fertilized or (2) an increase in pollen discounting and, therefore, a disproportionate decrease in male outcrossing success. Complete selfing is stable when selfing increases the proportion of ovules fertilized for all selfing rates. Stable selfing is zero or one in cases where the selfing rate has no effect on the proportion of ovules fertilized or when pollen discounting does not increase with selfing. Higher inbreeding depression tends to decrease the optimal selfing rate, and lower inbreeding depression (higher dominance coefficients and lower mutation rates) is more favorable to the existence of stable intermediate selfing rates. Approaches such as this that explicitly incorporate the interdependence of selfing, ovule fertilization, and male outcrossing may help explain the persistence of intermediate selfing rates in animal-pollinated plants.

Introduction

‘From the point of view of macroevolution, features of breeding systems and floral mode largely appear as ‘evolutionary noise,’ as they are highly labile, and attempts at character polarization and parsimony criteria would hardly make sense from this angle....Conversely, from the point of view of microevolution, breeding systems and floral modes are the central elements, while the bauplan is too static to be of relevance. Nevertheless, the spectrum of reproductive strategies in larger groups is not completely random but shaped by bauplan constraints to some extent...’

(Endress, 1994, p. 401)

The quotation above indicates that plants not only exhibit a great variety of breeding systems, but also that this variety is often distributed among recently evolved groups, such as populations, species, and genera. Plants, therefore, should be particularly appropriate for studying the selective forces acting on breeding systems, because these forces should be currently acting. In contrast, comparisons among higher taxonomic levels are more likely to reflect forces that are no longer acting (Williams, 1975). The great variety of breeding systems in plants is partly reflected in discrete phenotypic sexual morphs (hence hermaphroditism *sensu stricto*, monoecy, dioecy, gynodioecy, andromonoecy, and so forth.). Within this range, individuals of the vast majority of seed plants, perhaps 90%, possess both male and female sexual functions (Lloyd, 1983; Yampolsky & Yampolsky, 1922). These cosexual plants

exhibit a diversity of floral traits and associated mating systems, from complete outcrossing to complete self-fertilization.

Evolution of the selfing rate has recently received much theoretical attention (reviewed in Jarne & Charlesworth, 1993; Uyenoyama, Holsinger & Waller, 1993). There are two primary genetic factors guiding the evolution of selfing, and they work in opposition. A completely selfing genotype that suffers no loss of success through pollen export will invade an outcrossing population, because its genes are twice represented in its selfed seeds and once represented in seeds it has fertilized through pollen export (Fisher, 1941). This 150% transmission advantage has been termed the 'automatic selection advantage' of self-fertilization (Jain, 1976). The main genetic force opposing this automatic advantage is thought to be inbreeding depression, the reduced fitness of selfed relative to outcrossed progeny. Inbreeding depression is a ubiquitous feature of populations and has two possible genetic causes, both arising from the increase in offspring homozygosity caused by inbreeding as compared to outcrossing (Charlesworth & Charlesworth, 1987; Wright, 1977). The overdominance hypothesis proposes that inbreeding depression is caused by loci exhibiting heterozygous advantage. The partial dominance hypothesis holds that it results from continual mutation to partially recessive genes (Charlesworth & Charlesworth, 1987; Crow, 1993). Deleterious mutations of large effect are highly recessive, while those of small effect are more nearly additive and appear at much higher rates (Charlesworth & Charlesworth, 1987; Fu & Ritland, 1994; Houle et al., 1992; Johnston & Schoen, 1995; Simmons & Crow, 1977).

The genetic basis of inbreeding depression strongly affects its equilibrium level as a function of the selfing rate. When inbreeding depression is primarily caused by deleterious mutations, the level expressed upon self-fertilization is expected to decrease with the selfing rate (Lande & Schemske, 1985). The shape of the decrease depends primarily on the type of selection, dominance levels and mutation rates (Charlesworth, Morgan & Charlesworth, 1990, see Figure 1). When caused by overdominance, however, the equilibrium depression behaves quite differently, and can even rise with continued selfing (Charlesworth & Charlesworth, 1990). Deleterious mutations appear to be the primary cause of inbreeding depression (Charlesworth & Charlesworth, 1987; Johnston & Schoen, 1995; Lande & Schemske, 1985; Wright, 1977).

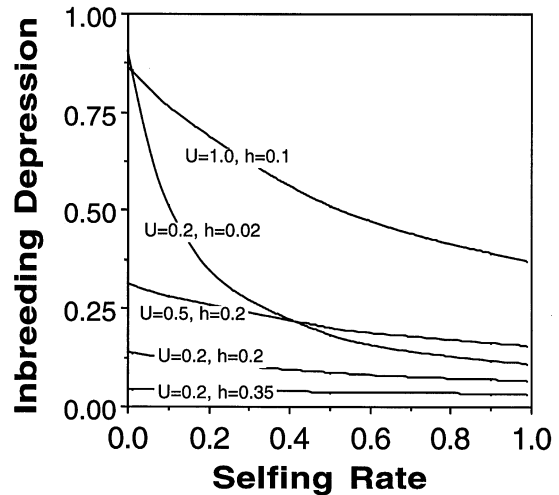


Figure 1. Inbreeding depression as a function of the population mean rate of self-fertilization when inbreeding depression is caused by deleterious mutations of dominance h occurring at the rate of U per diploid genome per generation. Mutations at different loci have multiplicative selective effects. Higher mutation rates and lower dominance coefficients cause lower equilibrium inbreeding depression. Lines represent polynomial regressions of results of Charlesworth, Morgan and Charlesworth (1990; see Appendix).

The strength and direction of selection on the selfing rate potentially depend on both genetic and ecological factors. Although genetic factors should apply to all organisms, ecological factors might be limited to particular situations or taxa. Therefore, perhaps in the interest of generality, models have historically emphasized genetics rather than ecology. 'Genetic' factors include inbreeding depression and the automatic selection advantage. 'Ecological' models consider when, how, and whether pollen is transferred. These two types of model are not mutually exclusive. To date, however, genetic models have considered few ecological details (pollen discounting is an exception), and ecological models have primarily investigated specific modes of pollen transfer occurring in the absence of other modes and ignored the evolution of inbreeding depression. Most models conclude that plants should optimally self-fertilize either all or none of their ovules, according to the level of inbreeding depression (Charlesworth, 1980b; Charlesworth, Morgan & Charlesworth, 1990; Lande & Schemske, 1985; Lloyd, 1979). Exceptions to this prediction are rather specialized and include inbreeding depression caused by heterozygote advantage (Campbell, 1986; Charlesworth & Charlesworth, 1990; Holsinger, 1988; Uyenoyama & Waller, 1991), differences in dispersal

ability of selfed and outcrossed offspring (Holsinger, 1986; Schoen & Lloyd, 1984), and lack of purging of inbreeding depression with continued inbreeding (Damgaard, Couvet & Loeschke, 1992; Latta & Ritland, 1993, 1994a).

Recent models incorporating pollination ecology have also identified optimal intermediate selfing rates under some conditions. These models have incorporated pollination ecology in various ways and with different genetical factors (Holsinger, 1991; Holsinger, 1992; Holsinger, 1996). Some models, for example, omit inbreeding depression (Holsinger, 1991), while others assume that inbreeding depression does not evolve with the selfing rate (Lloyd, 1992; Sakai, 1995). What remains is to study the effects of selfing on other aspects of pollen transfer when inbreeding depression is allowed to evolve in concert with the selfing rate.

For a population in mutation-selection equilibrium, inbreeding depression is determined primarily by the rate of appearance and dominance levels of deleterious mutations, while selection coefficients against homozygous mutants have negligible effects (Charlesworth, Morgan & Charlesworth, 1990, Figure 1). A mutant with an altered selfing rate is expected to experience the same inbreeding depression upon selfing as other members of the population. Its inbreeding depression is, therefore, a function, $\delta(\bar{S})$, of the mean selfing rate, \bar{S} . The mean selfing rate also determines the maximal success that the mutant can achieve through pollen export. Mean selfing rate thus plays a dual role in individual fitness. Consider a mutant with an increased selfing rate. All else being equal, it will be favored if inbreeding depression is less than one-half and selected against in the opposite case (Lloyd, 1979). All else, however, is not generally equal, particularly in sessile organisms that depend on external agents for gamete transfer. Instead, changes in selfing will be accomplished by alterations of the flower or inflorescence that affect other aspects of reproductive success. Thus, the fate of the mutant will depend on the average selfing rate (which will affect the level of inbreeding depression) and the way that the change in the selfing rate affects pollen export and pollen receipt. It seems most realistic to view the evolution of the selfing rate as the outcome of both direct and indirect selection, where indirect selection results from direct selection on female and male reproductive success. The dissection of total selection into direct and indirect parts has been developed for traits or fitness components (Lande & Arnold, 1983).

Although many plant species are self-incompatible and thus wholly outcrossing, most species are in fact partially self-fertilizing (Barrett & Eckert, 1990; Schemske & Lande, 1985). Therefore, either most populations are far from evolutionary equilibrium, which seems unlikely, or genetic models (those driven primarily by inbreeding depression) fail to capture an essential feature of plant reproduction (Schemske & Lande, 1985; Schemske & Lande, 1987; Waller, 1986). That inbreeding depression is not the whole story is supported by the lack of tight correlation between selfing rates and levels of inbreeding depression in closely related populations (Husband & Schemske, 1996; Johnston & Schoen, 1996; Latta & Ritland, 1994b). That inbreeding depression is nevertheless an important force is supported, first, by a trend toward bimodality in the distribution of selfing rates (Schemske & Lande, 1985) and, second, by the lower inbreeding depression in highly selfing than in highly outcrossing populations (Holtsford & Ellstrand, 1990; Husband & Schemske, 1996; Johnston & Schoen, 1996; Latta & Ritland, 1994b; Wright, 1977).

The present paper investigates the evolution of the rate of self-fertilization under different kinds of pollination ecology when inbreeding depression is allowed to evolve with the rate of self-fertilization. The models presented here separately consider two general situations: selfing rate is correlated with the proportion of ovules fertilized and selfing rate affects male outcrossing success through pollen export. Models are framed so as to be testable. Inbreeding depression is assumed to result from mutation to partially recessive, deleterious genes. Of particular interest are the general conditions sufficient for the evolution of optimal intermediate selfing rates and the relative importance of inbreeding depression and pollination ecology. It is concluded that pollination ecology will often cause intermediate levels of self-fertilization to be evolutionarily stable, and that the optimal level is influenced by inbreeding depression. The approach used in these models may help explain why many wind-pollinated species have selfing rates very near zero or one, while animal-pollinated species are often intermediate (Aide, 1986; Barrett & Eckert, 1990; Schemske & Lande, 1985).

General approach and rationale

The fitness of an individual cosexual plant with discrete generations is the sum of genetic contributions

to offspring achieved through selfed seed, outcrossed seed, and pollen export,

$$\text{Fitness} = 2[1 - \delta(\bar{S})]N_s + N_x + N_p,$$

where N_s , N_x and N_p are the number of ovules fertilized through selfing, outcrossing and pollen export, respectively, and $\delta(\bar{S})$ is inbreeding depression as a function of the population mean selfing rate, \bar{S} . $1 - \delta$ is the ratio of expected fitnesses from self-fertilization and outcrossing. Consider a rare mutant phenotype whose selfing rate differs from that of other members of the population. Its success through pollen export, N_p , is determined by whether the common phenotype makes ovules available for outcrossing and, if so, by the relative competitive abilities of the two phenotypes. Thus, the fitness, w , of the rare mutant is

$$w = 2[1 - \delta(\bar{S})]N_s + N_x + p\bar{N}_x, \quad (1a)$$

where p represents the proficiency of the focal phenotype at fertilizing ovules of other plants, relative to that of the common phenotype. Written in terms of the selfing rate, $S = N_s/(N_s + N_x)$, equation (1a) becomes

$$N_{tot}[1 + [1 - 2\delta(\bar{S})]S] + p\bar{N}_{tot}(1 - \bar{S}), \quad (1b)$$

where N_{tot} is total number of fertilized ovules of the mutant phenotype.

Most previous studies of selection on the selfing rate have assumed that selfing does not affect the number of ovules fertilized (Charlesworth, Morgan & Charlesworth, 1991; Charlesworth, Morgan & Charlesworth, 1990; Fisher, 1941; Lande & Schemske, 1985; Lloyd, 1979; Nagylaki, 1976). In such studies, the number of fertilized ovules was constant and any ovule not selfed was assumed outcrossed. There was thus a correlation of negative one between the numbers of selfed and outcrossed ovules. This perfect, negative correlation ignores the ecology of pollination; that is, it ignores the 'functional dimensions' (Lloyd, 1992). In animal-pollinated plants, the dispersal and receipt of pollen occur as a result of visits to flowers. The quantities of pollen dispersed, received, and deposited on self stigmas should be interrelated in ways that depend on the plants and animals involved. These three aspects of pollen transfer are, therefore, expected to be correlated events. It seems quite unlikely that available evolutionary pathways are constrained to an exact trade-off between number of selfed and outcrossed ovules.

Therefore, models making this assumption can be seen as studying selection on the selfing rate per se, with all else being equal.

Reproductive success achieved through each of the three pathways can be expressed as a function of the selfing rate or as a function of the number of selfed seeds. In terms of the selfing rate,

$$w = [1 + S(1 - 2\delta(\bar{S}))]f_{tot}(S) + \frac{f_p(S)}{f_p(\bar{S})}(1 - \bar{S})f_{tot}(\bar{S}). \quad (2a)$$

In describing particular ecological situations it is sometimes more useful to express fitness in terms of numbers of selfed ovules, so that

$$w = 2[1 - \delta(\bar{S})]N_s + F_x(N_s) + \frac{F_p(N_s)}{F_p(\bar{N}_s)}F_x(\bar{N}_s). \quad (2b)$$

(Note that lower-case [' f '] is used for functions of selfing rate per se, while upper-case [' F '] is used for functions of numbers of selfed ovules.) These two equivalent formulas permit investigation of optimal selfing under many ecological relationships between selfing, outcrossing, and pollen export.

In the models here, correlated evolution of the selfing rate and inbreeding depression is assumed to occur slowly, in small steps, such that they are in approximate equilibrium. Specific equilibrium levels of inbreeding depression as functions of the mean selfing rate were obtained by fitting fourth-degree polynomials to the values presented by Charlesworth, Morgan, and Charlesworth (1990) (see Figure 1). Regression coefficients are given in the Appendix. These authors investigated a range of mutation rates per diploid genome, U , and dominance coefficients, h , that cover much of the range of experimentally obtained estimates (Crow, 1993; Houle et al., 1992; Johnston & Schoen, 1995; Keightly & Caballero, 1997; Kondrashov, 1988). Their study assumed multiplicative selection (i.e., nonsynergistic effects of multiple mutations) and no linkage, but further studies found these assumptions to have little effect on the equilibrium level of inbreeding depression (Charlesworth, Morgan & Charlesworth, 1991; Charlesworth, Morgan & Charlesworth, 1992).

Evolutionarily stable selfing rates can be determined by examining differences in absolute fitness between any two levels of selfing. Such an analysis is equivalent to finding the maximum absolute fitness of a rare phenotype, for those cases where strategy does not change in response to frequency (Lloyd, 1983). This

seems reasonable in organisms with a limited behavioral repertoire. We, therefore, find the first partial derivative of fitness of the rare strategy with respect to its selfing rate analyzed at the mean. When the derivative is negative for all selfing rates, there is everywhere selection to decrease selfing, and then the optimal selfing rate is zero. When the derivative is positive for all selfing rates, then the optimal selfing rate is one. Threshold selfing rates occur when $\partial w/\partial S = 0$ at $S = \bar{S}$ and $\partial^2 w/\partial S^2 > 0$ at $S = \bar{S}$. There is selection to decrease selfing when the population is below the threshold and to increase selfing when above. Sufficient conditions for evolutionarily stable *intermediate* selfing rates, therefore, are that $\partial w/\partial S = 0$ at $S = \bar{S}$ and $\partial^2 w/\partial S^2 > 0$ at $S = \bar{S}$ for $0 < S < 1$ (2a) or $N_s, N_x > 0$ (2b).

Ovule fertilization and pollen export as functions of selfing rate

To study the effects of selfing rate on ovule fertilization and on pollen export, we can use the simple function

$$y = b + (m - b)S^c, \quad (3)$$

where y represents a fertility component, and b , c , and $m \geq 0$. In this study, y will be used to represent separately the proportion of ovules fertilized and pollen discounting (defined below). We also limit the values of b and m to 0 and 1, so that y is either an increasing ($b < m$) or decreasing ($b > m$) function of selfing, and both y and S are confined to the region between 0 and 1, inclusive (see Figure 2A - D). b is the y -intercept. (Cases where y reaches 0 or 1 at values other than complete selfing or outcrossing should also be studied (Figure 2E, F). Such nonmonotonic functions are easily accommodated by choosing values other than 0 or 1 for b , m , or both.) The parameter c determines curve shape. Curves are linear for $c = 1$. y is constant when $c = 0$ and would be indicated as a horizontal line in Figure 2, with height determined by m and b . y is also constant (at b) whenever $m = b$. Cases where $c > 1$ represent accelerating increases ($m > b$) or decreases ($m < b$) with selfing. Cases where $0 < c < 1$ represent *decelerating* increases ($m > b$) or decreases ($m < b$) with selfing.

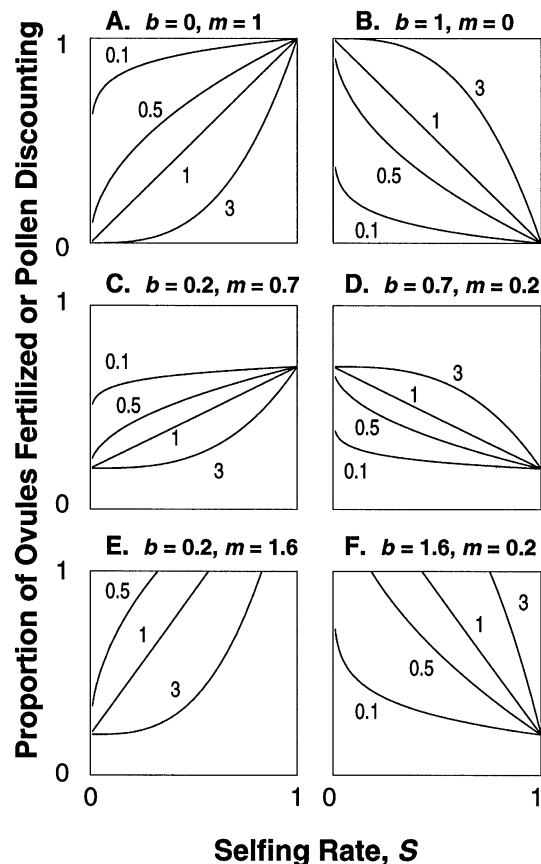


Figure 2. Simple function (equation 3) used to study the relations between rate of self-fertilization, S , and some aspect of reproductive success, y . In this study, y is the proportion of ovules fertilized or pollen discounting. y increases with selfing for $b < m$ and decreases for $b > m$. Graphs A - D represent monotonic increases of y with selfing, so that maximal and minimal y occur at opposite, extreme selfing rates. In E and F, complete y is reached at selfing rates below one. Values of c are shown on graphs.

Selfing affects success as a seed parent

In this section we consider cases where the rate of self-fertilization can affect the number of ovules that are fertilized. We first study the case where selfing rate has no effects on pollen export, so that conclusions can be based on ovule fertilization alone. Using (1) and (2), one can define a general relationship between the number of outcrossed (N_x) and selfed (N_s) ovules per plant: $N_x = F_x(N_s) = (1 - S)f_{tot}(S)$. This relationship is general such that it can specify that a change in the number of fertilized ovules of one cross type can have any effect, including none, on the number of the other type. In the absence of pollen discounting, the number of selfed ovules has no effect on relative proficiency

as a male outcrosser. Hence, throughout this section, $F_p(N_s)/F_p(\bar{N}_s) = 1$ and from (2)

$$w = [1 + S(1 - 2\delta(\bar{S}))]f_{tot}(S) + (1 - \bar{S})f_{tot}(\bar{S}) \quad (4a)$$

and

$$w = 2[1 - \delta(\bar{S})]N_s + F_x(N_s) + F_x(\bar{N}_s). \quad (4b)$$

The selfing rate is selected to increase when $\partial w/\partial S > 0$ (4a), and the number of selfed ovules is selected to increase when $\partial w/\partial N_s > 0$ (4b). A change in number does not necessarily imply a change in rate. For example, when increasing the number of selfed ovules causes an even greater increase in the number of outcrossed ovules, the selfing rate declines.

Assume that there is a maximum number of ovules, N_{max} , that can successfully be fertilized, such that $N_s + N_x \leq N_{max}$, and N_{max} does not vary with N_s . Because the sum of selfed and outcrossed ovules cannot exceed N_{max} , the relation $F_x(N_s)$ must lie on or to the left of a line having slope -1 and $F_x(0) = N_{max}$. As we are not considering the evolution of ovule number, this line segment connecting points (0, N_{max}) and (N_{max} , 0) is, therefore, a constraint. The region to the left of this line represents pollen limitation of seed production.

The proportion of ovules, $q(S)$, fertilized can be related to the selfing rate in a linear or nonlinear way and in an increasing or decreasing way by using (3), so that

$$q(S) = b_o + (m_o - b_o)S^{c_o} \quad (5)$$

where subscript 'o' refers to 'ovule' (see Figure 2). When selfing has no effect on pollen export, fitness is

$$\begin{aligned} w &= [1 + S - 2\delta(\bar{S})S][b_o + (m_o - b_o)S^{c_o}] + \\ &(1 - \bar{S})[b_o + (m_o - b_o)\bar{S}^{c_o}] \\ \frac{\partial w}{\partial S} \Big|_{S=\bar{S}} &= \frac{b_o[1 - 2\delta(\bar{S})\bar{S} + (b_o - m_o)\bar{S}^{c_o} + (1 + c_o)(2\delta(\bar{S}) - 1)\bar{S} - c_o]}{\bar{S}} \\ \frac{\partial^2 w}{\partial S^2} \Big|_{S=\bar{S}} &= c_o(b_o - m_o)[1 - c_o + [2\delta(\bar{S}) - 1] \\ &(1 + c_o)\bar{S}] \bar{S}^{c_o - 2} \end{aligned} \quad (6)$$

When $c_o = 0$ or $b_o = m$, the proportion of ovules fertilized is unaffected by selfing. In such cases intermediate selfing is never stable, as the partial second derivative is always zero. This recovers the most-widely studied

type of model. When c_o is zero, selfing is selected up or down according to the levels of b_o , m_o and inbreeding depression, which together form a threshold. When $b_o = m_o$, the evolutionary trajectory is determined by the relative values of b_o and inbreeding depression.

The values of b_o and m_o have straightforward biological meaning. Any situation where selfing increases the proportion of fertilized ovules is represented by $b_o < m_o$. Selfing is often considered an adaptation to augment seed production. Such 'reproductive assurance' is usually conceived of as autonomous selfing, but applies equally well to vector-mediated selfing. On the other hand, when $b_o > m_o$, the proportion of ovules fertilized decreases with the selfing rate. This can occur only if each added selfed ovule causes a decrease of greater than one of outcrossed ovules. Lloyd (1992) referred to the displacement of outcrossed ovules by selfing as 'seed discounting,' by analogy to pollen discounting. Seed discounting is in a sense the obverse of reproductive assurance.

Evolutionarily stable selfing rates were found by simultaneously solving $\delta = \delta(\bar{S})$ and $\partial w/\partial S|_{s=\bar{s}} = 0$ for \bar{S} .

Real solutions are thresholds of disruptive selection or optimal selfing rates according to whether the second partial derivative is positive or negative, respectively. Results are presented in Table 1 and Figure 3 (obtained using Mathematica[®] 3.0 [Wolfram Research, Inc.] when possible; otherwise approximate results are from graphs such as Figures 3 and 4). When selfing rate has no influence on the proportion of ovules fertilized ($c_o = 0$), evolutionarily stable selfing rates are 0 or 1, according to whether the level of inbreeding depression is greater or less than one-half, respectively (Table 1). This is the case most often studied (Charlesworth, Morgan & Charlesworth, 1990; Lande & Schemske, 1985). For the five combinations of h and U included here, two give sufficiently high inbreeding depression at low selfing rates to allow optimal selfing to be sometimes 0 (Table 1). These two cases recover the results of Lande and Schemske (1985) that historically outbreeding populations should experience high inbreeding depression upon selfing and should be selected to maintain complete outbreeding, while more-highly selfing populations will have diminished inbreeding depression and should become wholly selfing.

When the proportion of ovules fertilized climbs with the selfing rate ($b_o = 0, m_o = 1$) for all selfing rates, then the evolutionarily stable rate is one regard-

Table 1. Optimal rates of self-fertilization under mutation-selection balance when selfing affects the proportion of ovules fertilized. There is no effect on male outcrossing success (i.e., no pollen discounting). Threshold population mean selfing rates are given in parentheses below instances of more than one potential optimum. When population mean selfing is below (above) this threshold, the optimal selfing rate is given by the lower (higher) value. Parameters b_o , c_o and m_o are from equation (6) and are represented in Figure 2. Inbreeding depression tends to increase in columns from left to right (see Figure 1)

c_o	$U = 0.2$ $h = 0.35$	$U = 0.2$ $h = 0.2$	$U = 0.5$ $h = 0.2$	$U = 0.2$ $h = 0.02$	$U = 1$ $h = 0.1$
Fertilization proportion independent of S for all S ($c_o = 0, b_o = 1$)					
0	1	1	1	0,1 (0.12)	0,1 (0.52)
Fertilization proportion increases with S for all S ($b_o = 0, m_o = 1, c_o > 0$)					
> 0	1	1	1	1	1
Fertilization proportion decreases with S for all S ($b_o = 1, m_o = 0, c_o > 0$)					
0.1	0	0	0	0	0
0.5	0	0	0	0	0
1	0	0	0	0	0
2	0.32	0.29	0.21	0	0
3	0.45	0.43	0.38	0, 0.39 (0.12)	0
5	0.58	0.57	0.54	0, 0.55 (0.11)	0
10	0.72	0.72	0.71	0, 0.71 (0.11)	0

less of inbreeding depression, as the first derivative in (6) is positive (Table 1).

When the fertilization proportion decreases with selfing ($b_o = 1, m_o = 0$), stable selfing occurs at zero or an intermediate rate, and never at one. With a linear or decelerating decrease ($c_o \leq 1$), the first derivative (6) is never positive, so that optimal rates are zero (Table 1). When selfing causes the fertilization proportion to decline at accelerating rates ($c_o > 1$), the sign of the first derivative depends on the values of c_o and inbreeding depression. Increasing c_o results in higher optimal selfing. The effect of inbreeding depression is to decrease the optimal selfing rate, sometimes outside of the region where c_o has any effect, so that optimal selfing is zero (Figure 3, Table 1). Sometimes stable selfing occurs at zero and at an intermediate level. For example, when U is 0.2 and h is 0.02, optimal selfing should evolve to be zero if the population has a history of selfing below about 12%, but should evolve to an

intermediate level, depending primarily on c_o , if the population is historically more highly selfing (Table 1, Figure 3).

Selfing affects male outcrossing success (pollen export)

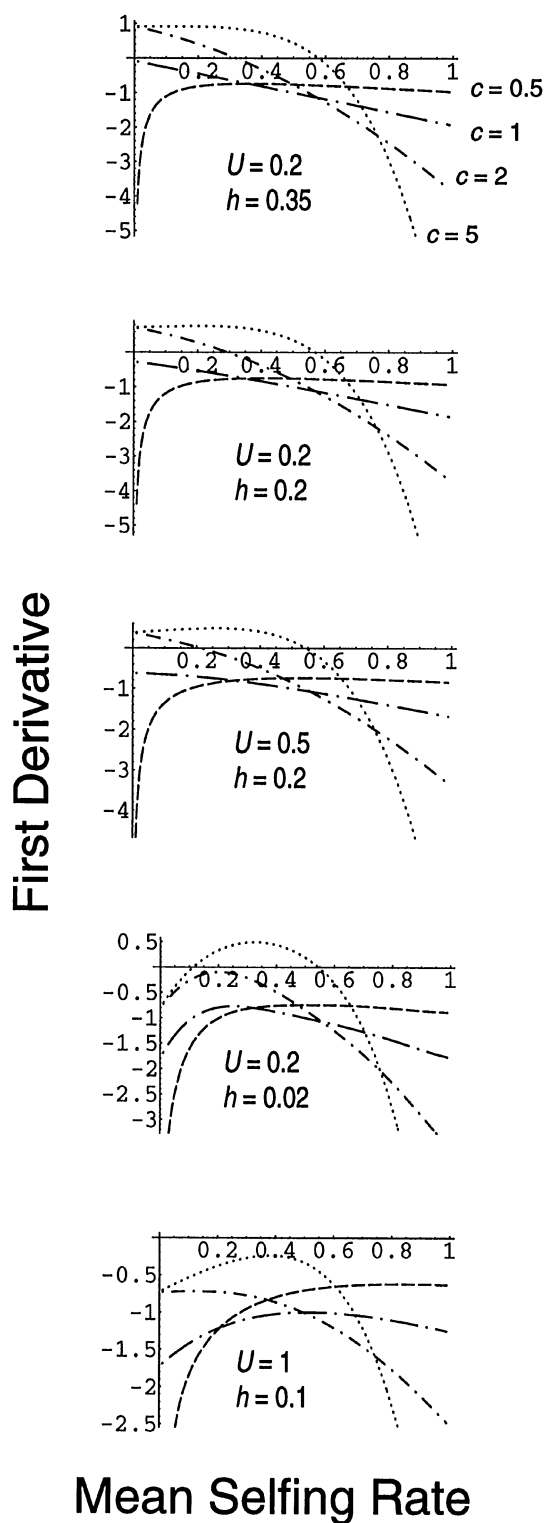
In this section we investigate the cases where rate of self-fertilization can affect an individual's ability to fertilize ovules of other plants. This may occur by the direct co-opting of pollen, by interfering with the pollen-removal process within flowers, or by pollen transfer among flowers on a plant (geitonogamy). Pollen discounting, D , refers to those cases where selfing reduces success through pollen export (Charlesworth, 1980b; Holsinger, Feldman & Charlesworth, 1984; Nagylaki, 1976). It is conveniently expressed as a fraction of the selfing rate (see Lloyd, 1992 for an alternative definition). The product DS ($0 \leq DS \leq 1$) is then the total reduction in pollen export expressed as a fraction of the case with no pollen discounting. Thus, when $D = 0$, selfing has no effect on outcross siring success. When $D = 1$, siring success is reduced by S to $1 - S$, the automatic transmission advantage of selfing disappears, and the level of selfing is selectively neutral.

The effects of pollen discounting on the optimal selfing rate have previously been analyzed for the case where it remains constant across all selfing rates (Charlesworth, 1980a; Lloyd, 1979). The model under those circumstances is

$$w = 1 + [1 - 2\delta(\bar{S})]S + (1 - \bar{S}) \frac{(1 - DS)}{(1 - D\bar{S})}, \quad (7)$$

where D is a constant ($0 \geq D \geq 1$). Constant pollen discounting causes the threshold level of inbreeding depression to be shifted from one-half to $0.5(1-D)/(1-D\bar{S})$ (Charlesworth, 1980b). That is, in the presence of pollen discounting ($D > 0$), increased selfing is selected whenever inbreeding depression is less than a threshold value that is below one-half. Pollen discounting reduces Fisher's (1941) automatic transmission advantage of selfing. With constant pollen discounting, stable optimal selfing rates are 0 or 1, as the second derivative is 0 (Charlesworth, 1980a; Lloyd, 1979).

It does not appear biologically necessary, or even likely, that pollen discounting remain constant for all levels of selfing. Instead, evolutionary changes in



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Figure 3. Effects of seed discounting (here $b_o = 1$, $m_o = 0$) on optimal selfing rates when there is no pollen discounting. First partial derivatives, evaluated at mean selfing, are shown as functions of population mean selfing for five combinations of mutation rate and dominance of deleterious mutations. When the derivative exceeds zero, there is selection to increase selfing; when it is less than zero, there is selection to decrease selfing. Lines crossing $\partial w / \partial S|_{s=\bar{s}} = 0$ with negative slope indicate stable intermediate selfing; positive slopes indicate thresholds. Numerical values of optima are in Table 1. Inbreeding depression tends to increase from the top graph to the bottom (Figure 1). The parameters m_o , b_o , and c_o are explained in the text and in Figure 2.

the selfing rate may be accomplished in ways that increase or decrease pollen discounting (Holsinger, 1992). These two possibilities can be studied by making discounting a function, $D(S)$, of the selfing rate, so that

$$w = 1 + [1 - 2\delta(\bar{S})]S + (1 - \bar{S}) \left(\frac{1 - SD(S)}{1 - \bar{S}D(\bar{S})} \right). \quad (8)$$

Again we can use (3) for a simple model that allows pollen discounting to remain constant or change in a linear, concave or convex manner with the selfing rate:

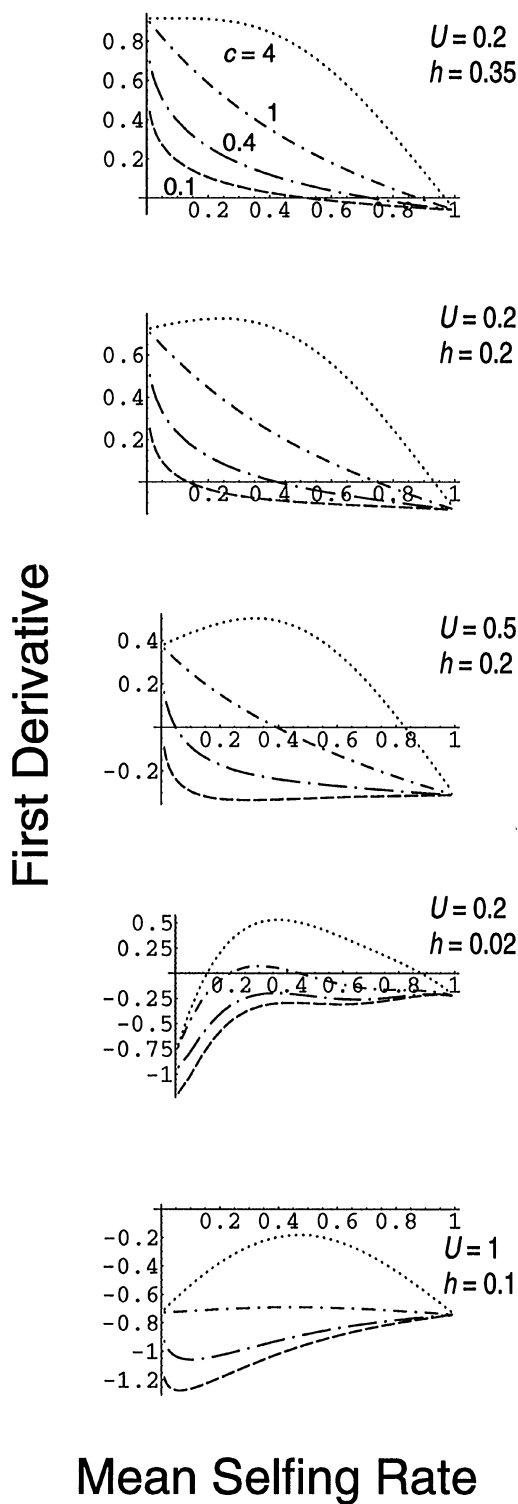
$$D(S) = b_p + (m_p - b_p)S^{c_p}, \quad (9)$$

where m , $b \geq 0$. Pollen discounting increases with the selfing rate whenever $m_p > b_p$ (Figure 2A, C, E) and decreases whenever $m_p < b_p$ (Figure 2B, D, F). The case of constant discounting is recaptured when $c_p = 0$, or $b_p = m_p$, and would be indicated as a horizontal line in Figure 2, with height determined by b_p and m_p .

When pollen discounting changes with the selfing rate according to equation (9), then

$$\begin{aligned} \frac{\partial w}{\partial S} \Big|_{S=\bar{S}} &= 1 - 2\delta(\bar{S}) + \frac{(1-\bar{S})[(1+c_p)(b_p-m_p)\bar{S}^{c_p} - b_p]}{1-\bar{S}[b_p+(m_p-b_p)\bar{S}^{c_p}]} \\ \frac{\partial^2 w}{\partial S^2} \Big|_{S=\bar{S}} &= \frac{c_p(1+c_p)(b_p-m_p)(1-\bar{S})\bar{S}^{c_p-1}}{1-\bar{S}[b_p+(m_p-b_p)\bar{S}^{c_p}]} \end{aligned} \quad (10)$$

Thus, whenever pollen discounting diminishes as selfing increases (i.e., $b_p > m_p$), the second derivative in (10) is positive, so that evolutionary equilibrium is achieved at complete selfing or complete outcrossing, as with constant discounting discussed above (Table 2). The levels of inbreeding depression and discounting together then determine whether complete selfing or outcrossing is evolutionarily stable. The



←

Figure 4. Effects of pollen discounting (here $b_p = 0$, $m_p = 1$) on optimal selfing rates when selfing does not affect the proportion of ovules fertilized. Numerical values of optima are in Table 2. For further explanation, see legend of Figure 3.

effect of increasing either c_p or inbreeding depression is to increase the threshold mean selfing rate, above which the optimal selfing rate is one and below which it is zero (Table 2).

In contrast to the results above, whenever pollen discounting increases with the selfing rate (i.e., $b_p < m_p$), the second derivative is negative. As a result, if the point where δ , b_p , c_p , and m_p satisfy $\partial w / \partial S|_{S=\bar{S}} = 0$ occurs at rates between zero and one, then there is an evolutionarily stable intermediate selfing rate (Figure 4). Table 1 shows that intermediate rates are the rule under these conditions, except where inbreeding depression is high. The effect of higher inbreeding depression is to lower the optimal selfing rate. As c_p increases, pollen discounting decreases (at least until $S = 1$), so that a higher level of selfing is required to cause the same discounting (Figure 4). This helps explain the rising optimal selfing rate with c_p in Table 1 when $b_p < m_p$.

In general, then, when pollen discounting is constant or decreases with the selfing rate, the optimal level of self-fertilization is zero or one, according to the level of inbreeding depression. When pollen discounting increases with the selfing rate for all S between zero and one, however, then an intermediate level of selfing is almost always optimal.

Discussion

The models investigated here are an incomplete set of all possibilities. Nevertheless, they are sufficiently general to cover many specific cases. An attempt was made to construct simple, general relations between the three aspects of reproductive success, rather than to investigate specific modes of pollination (Lloyd, 1979; Lloyd, 1992; Lloyd & Schoen, 1992). The relationships should be obtainable for real organisms. Interesting results on the relationships between the aspects of reproductive success have been obtained in experimental arrays (Harder & Barrett, 1995), and with the use of highly polymorphic genetic markers, such as short sequence repeats, individual selfing rates and possibly male outcrossing success should be obtainable in natural populations. To relate data to models, success

Table 2. Optimal rates of self-fertilization under mutation-selection balance when selfing affects male outcrossing success through pollen discounting but not the proportion of ovules fertilized. Threshold population mean selfing rates are given in parentheses below instances of more than one potential optimum (see heading of Table 1). Parameters b_p , c_p and m_p are from equation (9) and are represented in Figure 2. Inbreeding depression tends to increase in columns from left to right (see Figure 1)

c_o	$U = 0.2$ $h = 0.35$	$U = 0.2$ $h = 0.2$	$U = 0.5$ $h = 0.2$	$U = 0.2$ $h = 0.02$	$U = 1$ $h = 0.1$
Complete pollen discounting: independent of S for all S ($c_p = 0, b_p = 1, m_p = 1$)					
0	0,1 (0.24)	0,1 (0.32)	0,1 (0.04)	0,1 (0.18)	0,1 (0.53)
Pollen discounting increases with S for all S ($b_p = 0, m_p = 1, c_p > 0$)					
0.1	0.25	0.02	0	0	0
0.5	0.76	0.52	0.10	0	0
0.9	0.86	0.71	0.33	0, 0.40 (0.23)	0
1	0.88	0.75	0.39	0, 0.45 (0.20)	0
2	0.94	0.87	0.67	0, 0.70 (0.12)	0
3	0.96	0.91	0.78	0, 0.83 (0.11)	0
5	0.97	0.95	0.86	0, 0.91 (0.11)	0
10	0.99	0.97	0.93	0, 0.95 (0.11)	0, 0.74 (0.54)
Pollen discounting decreases with S for all S ($b_p = 1, m_p = 0$)					
0.1	1	1	1	0, 1 (0.13)	0, 1 (0.51)
0.4	1	0, 1 (0.02)	0, 1 (0.10)	0, 1 (0.18)	0, 1 (0.49)
0.5	1	0, 1 (0.03)	0, 1 (0.13)	0, 1 (0.19)	0, 1 (0.49)
0.9	0, 1 (0.03)	0, 1 (0.10)	0, 1 (0.22)	0, 1 (0.24)	0, 1 (0.51)
1	0, 1 (0.04)	0, 1 (0.11)	0, 1 (0.23)	0, 1 (0.25)	0, 1 (0.51)
2	0, 1 (0.16)	0, 1 (0.25)	0, 1 (0.37)	0, 1 (0.35)	0, 1 (0.57)
3	0, 1 (0.26)	0, 1 (0.35)	0, 1 (0.45)	0, 1 (0.44)	0, 1 (0.61)
5	0, 1 (0.41)	0, 1 (0.48)	0, 1 (0.56)	0, 1 (0.56)	0, 1 (0.68)
10	0, 1 (0.61)	0, 1 (0.65)	0, 1 (0.70)	0, 1 (0.70)	0, 1 (0.77)

through each of the three aspects of pollination should be expressed on a per-flower or per-inflorescence basis, as the present models treat flower number as equal in all phenotypes.

The parameter values studied here did not allow male outcrossing success to increase with any degree of self-fertilization. That is, pollen discounting increased, decreased, or remained constant with selfing, but any pollen discounting incurred a cost to male export success. It will be important to study cases where male success increases with selfing. This could be explored in the present models by making pollen discounting a negative function of the selfing rate (for example, $b_p = 0, m_p = -1$). Both conceptually and arithmetically, it is more straightforward, however, to make the relevant portion of male fertility [f_p from equation (2)] directly a function of selfing by using equation (8). Expressing the effects of selfing on male success directly, rather than through pollen discounting, simplifies comparison to the effects of selfing on seed production. Furthermore, it may often be more experimentally feasible to study success through pollen export than to measure pollen discounting. The models of pollen discounting can easily be transformed to male outcrossing success. When $b_p = 0$ and $m_p = 1$, pollen discounting is $D(S) = S^c$ and the relevant portion of male outcrossing success is $f_p(S) = 1 - S^{c+1}$ (equations 2a, 4). When $b_p = 1$ and $m_p = 0$, pollen discounting is $D(S) = 1 - S^c$ and male success is $f_p(S) = 1 - S + S^{c+1}$.

It was found that intermediate levels of selfing are evolutionarily stable for wide ranges of conditions. This conclusion is not reached in previous models that treated pollen discounting as constant or nonexistent and/or that considered selfing to have no effect on the total number of fertilized ovules. Holsinger (1991, 1992, 1996) has recently stressed the need for models that incorporate the realities of pollen transfer and has, therefore, developed the 'mass-action' approach. The mass-action model emphasizes pollen transfer and concludes that optimal intermediate selfing rates exist under a wide range of conditions. In these regards, the models studied here have similarities with the mass-action model. Mass-action models, however, do not incorporate inbreeding depression. Sakai (1995) has studied the effects of allocation to pollinator attraction. In a model with nonevolving inbreeding depression, he found optimal intermediate selfing rates when there are nonlinear constraints on flower number.

A plant realizes maximum reproductive success by optimizing each of the three aspects of reproduction:

selfed ovules, outcrossed ovules, and fertilized ovules of other individuals. In the present study, the cases of male outcrossing success and numbers of fertilized ovules were analyzed separately. That is, selfing affected either male success or numbers of ovules fertilized with the other being held constant. It would be more realistic to allow any correlations among numbers of selfed ovules, outcrossed ovules and fertilized ovules of other plants. This extension can easily be accomplished using equation (2) (to be presented). When sessile organisms depend on external agents, particularly when these are animals, we expect that maximizing reproductive fitness will often entail an intermediate selfing rate. The optimal selfing rate will be influenced by the relative fitness of selfed and outcrossed offspring and, therefore, by the rate of appearance and dominance levels of deleterious mutations.

Acknowledgements

I am grateful to R. Lande for the opportunity to discuss some of these issues in a rain of gannet guano, and I thank an anonymous reviewer for comments on the manuscript. Supported by grants from NSERC.

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Appendix

Table A1. Equilibrium inbreeding depression, $\delta(\bar{S})$, as a function of the average selfing rate \bar{S} , for different rates of mutation U , to alleles with dominance coefficient h . Constants β_0 to β_4 are polynomial regression coefficients for

the equation $\delta(\bar{S}) = \sum_{i=0}^4 \beta_i \bar{S}^i$ and were obtained by polynomial regression

from results of Charlesworth, Morgan & Charlesworth (1990).

U	h	β_0	β_1	β_2	β_3	β_4
0.2	0.02	0.90219	-4.7365	11.685	-12.534	4.7961
0.2	0.2	0.13864	-0.16488	0.14371	-0.52215	0
0.2	0.35	0.041903	0.0012764	-0.053668	0.070682	-0.029252
0.5	0.2	0.31203	-0.30677	0.16329	0.034146	-0.048115
1.0	0.1	0.86407	-1.0422	0.81163	-0.28076	0.017705