Evolution, 58(9), 2004, pp. 1924–1935

ON THE EVOLUTIONARY COSTS OF SELF-INCOMPATIBILITY: INCOMPLETE REPRODUCTIVE COMPENSATION DUE TO POLLEN LIMITATION

MARIO VALLEJO-MARÍN[1,2] AND MARCY K. UYENOYAMA[1,3]
[1]*Department of Biology, Box 90338, Duke University, Durham, North Carolina 27708-0338*
[2]*E-mail: mv6@duke.edu*
[3]*E-mail: marcy@duke.edu*

Abstract.—Pollen limitation affects plants with diverse reproductive systems and ecologies. In self-incompatible (SI) species, pollen limitation may preclude full reproductive compensation for prezygotic rejection of pollen. We present a model designed to explore the effects of incomplete reproductive compensation on evolutionary changes at a modifier locus that regulates the level of SI expression. Our results indicate that incomplete reproductive compensation greatly increases the evolutionary costs of SI, particularly in populations with low *S*-allele diversity. The evolutionary fate of modifiers of SI expression depends on the rate at which they are transmitted to future generations as well as the effects of SI on offspring number and quality. Partial SI expression can represent a stable condition rather than an evolutionarily transient state between full expression and full suppression. This unanticipated result provides the first theoretical support for the evolutionary stability of such mixed mating systems, the existence of which has recently been documented.

Key words.—Inbreeding depression, mating systems, pollen limitation, reproductive compensation, self-incompatibility.

Received April 28, 2004. Accepted June 2, 2004.

Hermaphroditic flowering plants exhibit a wide variety of self-incompatibility (SI) mechanisms, the best known of which inhibit fertilization by pollen that express genetically encoded specificities in common with the pistil. Pollen specificity is determined by the *S*-locus genotype of individual pollen grains or tubes in gametophytic SI (GSI) systems, and of the pollen parent in sporophytic SI (SSI) systems. Viewed as a form of parental manipulation, SI constitutes a mechanism for the preferential support of outbred offspring. Under pollen limitation (receipt of fewer compatible pollen grains than sufficient to fertilize all available ovules), this manipulation may incur substantial evolutionary costs.

Pollen Limitation Disfavors Self-Incompatibility

Parental manipulation incurs greater evolutionary costs as reproductive compensation declines. Fisher's (1930) concept of reproductive compensation refers to the recouping of parental investment in inviable or less favored offspring and its redirection toward new reproduction. In the context of sex-ratio evolution, for example, complete reproductive compensation implies manipulation of brood sex ratio independently of brood size. In the canonical model of SI (Wright 1939), rejection of incompatible pollen entails no reduction in seed set (complete reproductive compensation). In pollen-limited conditions, however, SI expression may incur direct declines in seed set.

Bateman (1948) suggested that mate availability rarely limits female reproduction in animals; in plants, however, pollen availability may constitute a major limitation of reproductive success through female function (reviewed by Burd 1994; Larson and Barrett 2000; Wilcock and Neiland 2002). Burd's (1994) survey of 258 species representing 77 angiosperm families showed that 62% suffer from pollen-limited seed set in at least one geographical location or one reproductive season. Various factors, including low levels of pollen production, pollinator availability, or mate availability, may contribute to pollen limitation (Wilcock and Neiland 2002). An overabundance of heterospecific pollen may interfere with the receipt of or fertilization by compatible pollen grains (Fishman and Wyatt 1999; but see Shore and Barrett 1984). Although limited pollen transfer is often associated with animal vectors, recent work indicates that it may be important in wind-pollinated species as well (Koenig and Ashley 2003). Theoretical studies suggest that pollen limitation may influence the evolution of various aspects of mating systems, including allocation to male and female function (e.g., Lloyd 1987; Olivieri et al. 1994; Maurice and Fleming 1995; Sakai 1995) or synchronization of reproduction (Satake and Iwasa 2002).

Pollen limitation is considered to be a condition favoring the evolution of self-compatibility (SC) from SI ancestors (Baker 1955; Charlesworth and Charlesworth 1979; Reinartz and Les 1994; Goodwillie 1999). Even under levels of deposition of conspecific pollen sufficient to ensure full seed set in SC plants, the expression of SI may reduce reproduction if a substantial fraction of the pollen received is incompatible. SI expression may inhibit reproduction within local neighborhoods of structured populations (Levin 1989; Byers and Meagher 1992; Goodell et al. 1997). Even after deposition, self pollen may directly interfere with outcross pollen by reducing germination rates or pollen tube growth (Ockendon and Currah 1977; Bertin and Sullivan 1988; Galen et al. 1989; Ramsey and Vaughton 2000) or promoting flower abscission or fruit abortion (Becerra and Lloyd 1992; Morse 1994; Vogler and Stephenson 2001). Larson and Barrett (2000) showed, using phylogenetically independent contrasts, that SI species show significantly greater gains in fruit set under experimental pollen supplementation than do SC species. To the extent that it reduces reproductive compensation, pollen limitation may substantially increase the evolutionary costs of SI.

Vekemans et al. (1998) conducted numerical simulations to explore the effect of fecundity selection on the evolution

of the *S*-locus. They incorporated fecundity selection into their models by limiting the number of pollen grains sampled before an unfertilized ovule was discarded. Under restriction to sampling of a single pollen grain, this formulation recovers Finney's (1952) zygote elimination model, which is equivalent to symmetric overdominance in viability with lethal homozygosis. As the maximum number of pollen grains sampled becomes arbitrarily large, their model converges to the standard Wright (1939) model, which prescribes no fecundity selection (complete reproductive compensation). They found that fecundity selection increases the number of segregating *S*-alleles and decreases their average life spans in finite populations, particularly in SSI systems.

Genetic Mechanisms of Self-Incompatibility Breakdown

Pollen limitation may promote evolutionary transitions from SI to SC. de Nettancourt (1977) recognized three principal genetic mechanisms for the breakdown of SI: (1) polyploidization or gene duplication; (2) mutations at the *S*-locus; and (3) mutations at other loci.

Polyploidization has long been recognized as a primary pathway to self-compatibility (Lewis 1943; Stebbins 1955). Transformation studies in model systems of both GSI and SSI have provided direct experimental support for this view. Indeed, the almost complete suppression of pistil specificity in transformants bearing an additional copy of part of the *S*-locus (Murfett et al. 1992; Conner et al. 1997) posed a major technical obstacle to the identification of the gene that encodes pistil specificity (Lee et al. 1994; Murfett et al. 1994; Takasaki et al. 2000). To investigate the determination of pollen specificity in *S*-RNase-based GSI, Golz et al. (1999) generated by X-irradiation of *Nicotiana alata* pollen grains mutations that impaired the pollen but not the pistil component of the SI rejection reaction. All mutations have now been shown to involve duplications, including some that lack the *S-RNase* gene, the product of which inhibits the growth of incompatible pollen tubes (Golz et al. 2001).

Mutations within the *S*-locus itself may contribute to SC by disrupting the expression or activity of products of the *S*-locus. For example, Royo et al. (1994) showed that an *S-RNase* allele isolated from a partially SC natural population of *Lycopersicon peruvianum* (Rick 1986) bore a point mutation that causes an amino acid substitution in the active site for RNase activity.

Modulators of SI expression segregating at loci distinct from the *S*-locus have been detected in many plants spanning a wide taxonomic range (reviewed by Levin 1996). Levin (1996) has suggested that many transitions from SI to SC may have arisen through genetic changes at modifier loci rather than at the *S*-locus itself. The modulation of *S*-locus expression by unlinked genes has been characterized in model systems of both SSI and GSI (reviewed by McClure et al. 2000; Cruz-García et al. 2003). In the form of SSI expressed in *Brassica*, modifiers have been described that induce suppression of the stigmatic rejection reaction, with (Nasrallah et al. 1992) or without (Ikeda et al. 1997) changing expression levels of *S*-locus-specific glycoproteins. In *N. alata*, which expresses *S*-RNase-based GSI, antisense suppression of a gene encoding a small protein (HT) suppresses *S*-allele-specific pollen rejection without affecting S-RNase expression (McClure et al. 1999). Analysis of a naturally occuring SC variant in *Petunia axillaris* indicated suppression of transcripts of a specific *S*-allele by a factor segregating at a distinct locus (Tsukamoto et al. 2003).

An increasing body of evidence suggests that rejection of incompatible pollen represents a quantitative, rather than qualitative, trait. Plants derived from natural *Phlox* populations responded rapidly to two cycles of artificial selection favoring increases or decreases in autogamous fruiting (Bixby and Levin 1996). Good-Ávila and Stephenson (2002) detected heritable variation for increasing self-fertility with flower age in *Campanula rapunculoides*. Stephenson et al. (2003) showed that the fraction of pollen tubes that succeeded in traversing the entire length of the style within 48 h of application of self pollen increased with flower age in *Solanum carolinense*; the finding of significant differences among genets may indicate genetic variation for reduced SI expression with age.

Evolutionary Modification of Self-Incompatibility Expression

Here we explore the evolutionary consequences of incomplete reproductive compensation on the level of expression of GSI. We study the evolutionary dynamics at a modifier locus, unlinked to the *S*-locus, which influences the proportion of pollen tubes screened for compatibility. For example, such a locus might influence the age of onset of SI breakdown in *C. rapunculoides* or *S. carolinense*. We find that in addition to substantially increasing the evolutionary costs of SI, incomplete reproductive compensation can give rise to evolutionarily stable states of partial SI expression.

Model

Our model describes evolutionary changes at a modifier locus that influences the level of expression of GSI in a population subject to incomplete reproductive compensation for rejected incompatible pollen. We assume that n functionally equivalent *S*-alleles segregate in the population in equal frequencies ($1/n$). Each of the n genotypes homozygous for an *S*-allele occurs with frequency u_0, and each of the $\binom{n}{2}$ heterozygous genotypes with frequency u_1. Let s denote the fraction of pollen deposited on a stigma that was produced by the same plant (self pollen) and σ the rate at which inbred zygotes (derived from self pollen) survive to reproduction relative to outbred zygotes ($0 \le s$, $\sigma \le 1$). Reproductive compensation influences the evolutionary dynamics through c, representing the seed set of fully self-incompatible relative to fully self-compatible plants ($0 \le c \le 1$).

Resident Population

In the resident population (prior to the introduction of variation at the modifier locus), all individuals screen a fraction t_0 of pollen received for *S*-allele compatibility, with all *S*-alleles within the complementary pollen fraction ($1 - t_0$) accepted. Within the screened fraction, each individual homozygous at the *S*-locus rejects pollen that express its single *S*-allele (proportion $1/n$), and each *S*-locus heterozygote rejects pollen bearing either of two *S*-alleles (proportion $2/n$).

Explicitly, the fractions of pollen accepted by *S*-locus homozygotes and heterozygotes correspond to $[(1 - t_0) + t_0(1 - s)(1 - 1/n)]$ and $[(1 - t_0) + t_0(1 - s)(1 - 2/n)]$. These expressions represent the average probability of acceptance over the phases in which screening is turned off $(1 - t_0)$ and on (t_0).

Reproductive compensation ameliorates the reduction in seed set expected to accompany rejection of incompatible pollen, with complete compensation implying no reduction. We incorporate this phenomenon into our model by setting the numbers of zygotes produced by homozygotes and heterozygotes equal to scaled fractions of accepted pollen, $[(1 - t_0) + t_0(1 - s)(1 - 1/n)]/d_0$ and $[(1 - t_0) + t_0(1 - s)(1 - 2/n)]/d_1$, for scaling factors given by

$$d_0 = (1 - c) + c[(1 - t_0) + t_0(1 - s)(1 - 1/n)] \quad (1a)$$

and

$$d_1 = (1 - c) + c[(1 - t_0) + t_0(1 - s)(1 - 2/n)]. \quad (1b)$$

Under full compensation ($c = 1$), this construction determines independence between seed set and *S*-locus genotype or level of pollen rejection, as in Wright's (1939) classic GSI model. Under incomplete compensation ($c < 1$), seed set declines with increases in SI expression level (t_0).

Genotypic frequencies in the offspring generation correspond to

$$Tu_0' = (1 - t_0)\{u_0^*[s\sigma + (1 - s)/n] + (n - 1)u_1^*[s\sigma + 2(1 - s)/n]/4\} \quad (2a)$$

and

$$Tu_1' = (1 - t_0)u_1^*[s\sigma + 2(1 - s)/n]/2 + 2(1 - s)[u_0^* + (n - 2)u_1^*/2]/n, \quad (2b)$$

in which the prime denotes frequencies among offspring, T ensures that the genotypic frequencies sum to unity, and

$$u_0^* = u_0/d_0 \quad \text{and} \quad (3a)$$

$$u_1^* = u_1/d_1, \quad (3b)$$

for d_0 and d_1 defined in equation (1a,b). Prior to the introduction of variation at the modifier locus, the frequencies of *S*-locus homozygotes and heterozygotes correspond to the single point $(\hat{u}_0, \hat{u}_1)$ that satisfies the condition for equilibrium $(u_0' = u_0, u_1' = u_1)$.

Modifier of Gametophytic Self-Incompatibility Expression

A modifier locus, which undergoes recombination with the *S*-locus at rate r, controls the level of expression of GSI. In the resident population, all individuals bear genotype M_0M_0 at the modifier locus and screen a proportion t_0 of pollen received for compatibility. Upon the introduction of modifier allele M_1, genotypes M_0M_1 (which screens a proportion t_1 of pollen received) and M_1M_1 (t_2) arise.

Assumption of complete symmetry among *S*-allele frequencies and genotypes permits reduction of the number of distinct genotypic frequencies to six. Let v_0 represent the frequency of M_0M_1 individuals homozygous for any particular *S*-allele among the n segregating in the population, and v_1 heterozygous for any pair of *S*-alleles; similarly, w_0 and w_1 represent the frequencies of M_1M_1 individuals that bear one or two distinct *S*-alleles $[n(u_0^A + v_0 + w_0) + \binom{n}{2}(u_1 + v_1 + w_1)] = 1$. All genotypes make equal contributions to the outcross pollen pool, of which M_0 pollen constitutes the fraction

$$p = n(u_0 + v_0/2) + \binom{n}{2}(u_1 + v_1/2) \quad (4)$$

and M_1 pollen the fraction q $(=1 - p)$. As in the resident population (eq. 3), the level at which each genotype expresses SI (t_i) influences its seed set. A full set of recursions appears in Appendix 1.

Results

Analysis

Restriction to free recombination ($r = 1/2$) and complete dominance of M_1 ($t_1 = t_2$) permits reduction of the number of free variables from four to two:

$$\gamma_0 = w_0 + v_0/2 \quad \text{and} \quad (5a)$$

$$\gamma_1 = w_1 + v_1/2. \quad (5b)$$

We made a further change in basis, to the biologically meaningful variables

$$q = n\gamma_0 + \binom{n}{2}\gamma_1 \quad \text{and} \quad (6a)$$

$$\delta = u_0\gamma_1 - u_1\gamma_0, \quad (6b)$$

the frequency of modifier allele M_1 and a measure of disequilibrium between the *S*-locus and the modifier locus, respectively. Positive δ $(\gamma_1/\gamma_0 > u_1/u_0)$ corresponds to a positive association between *S*-locus heterozygosity and the rare modifier allele.

We studied the analytical condition for the initial increase of a dominant modifier allele M_1 of weak effect (t_2 close to t_0) at a locus unlinked to the *S*-locus (Appendix 2). Using a modified form of local stability analysis (Uyenoyama 1991), we determined the relationship of the change over a single generation in the frequency of a modifier allele initiated in a particular genotypic configuration to the asymptotic behavior of the linearized system after a general local perturbation. To explore the evolutionary behavior of the system in the absence of some of these simplifying assumptions, we also conducted numerical iterations of the full recursion system.

Change over a Single Generation

Components of selection

From the multidimensional description of the linearized evolutionary dynamics (eq. A8), we obtained an expression for a one-generation change in gene frequency:

$$-T\Delta q = \frac{q(t_0 - t_2)}{2}\left[nu_0\frac{dR_0}{dt_0} + \binom{n}{2}u_1\frac{dR_1}{dt_0}\right] + n\binom{n}{2}\frac{(R_0 - R_1)\delta}{2}, \quad (7)$$

in which R_0 and R_1 represent rates of transmission of modifier alleles through *S*-locus homozygotes and heterozygotes:

$$R_0 = \{(1 - t_0)[s\sigma + (1 - s)/2n] + (1 - s)(1 - 1/n)/2\}/d_0 \quad \text{and} \tag{8a}$$

$$R_1 = \{(1 - t_0)[s\sigma + (1 - s)/n] + (1 - s)(1 - 2/n)/2\}/d_1. \tag{8b}$$

These measures of reproductive success at the modifier locus correspond to expected numbers of surviving offspring, weighted by parent-offspring relatedness with respect to the modifier locus. For example, the number of zygotes produced by selfing is proportional to $(1 - t_0)s$; although inbreeding depression reduces the viability of inbred offspring (σ), relatedness with respect to the modifier locus of parents to inbred offspring exceeds that to outbred offspring by twofold (1 to 1/2).

Two components of selection determine the fate of rare modifiers of SI expression. The first term on the right side of (7) represents the direct effect of changes in the level of expression of SI on expected rates of transmission of modifier alleles, and the second term selection pressures mediated by genetic associations between the modifier locus and the *S*-locus. We refer to these components as the direct and disequilibrium effects, respectively.

Direct effect

To determine whether SI expression promotes transmission of modifier alleles, we examined the nature of the dependence of R_0 and R_1 on t_0. Higher levels of SI expression increase transmission of modifier alleles through *S*-locus homozygotes ($dR_0/dt_0 > 0$) only if inbred offspring have sufficiently low viability:

$$\frac{1}{2} - \frac{(1 - c)[s + (1 - s)/n]}{2s\{1 - c[s + (1 - s)/n]\}} > \sigma. \tag{9}$$

SI expression promotes transmission of modifier alleles through *S*-locus heterozygotes ($dR_1/dt_0 > 0$) under a similar but more restrictive condition (eq. 9 with $1/n$ replaced by $2/n$).

Condition (9) indicates that under full reproductive compensation ($c = 1$), rejection of incompatible pollen improves transmission of modifier alleles only under a greater than twofold difference in viability between inbred and outbred offspring ($1/2 > \sigma$). The evolutionary costs of SI expression increase as the level of reproductive compensation (c) or number of segregating *S*-alleles (n) declines.

Disequilibrium effect

Because SI expression entails the rejection of only one pollen specificity by *S*-locus homozygotes and two by heterozygotes, homozygotes tend to transmit modifier alleles at higher rates:

$$R_0 - R_1 = \frac{t_0(1 - s)[1 - c + cs(1 - t_0)(1 - 2\sigma)]}{2nd_0d_1}. \tag{10}$$

In the absence of SI expression or outcross pollen ($t_0[1 - s] = 0$), *S*-locus homozygotes and heterozygotes transmit modifier alleles at equal rates ($R_0 = R_1$); otherwise, heterozygotes provide a less favorable conduit for modifier alleles ($R_0 > R_1$) under sufficiently low inbred viabilities:

$$\frac{1}{2} + \frac{1 - c}{2cs(1 - t_0)} > \sigma. \tag{11}$$

In particular, a twofold reduction in viability of inbred offspring ($1/2 \geq \sigma$) is sufficient to ensure higher rates of transmission of modifier alleles through *S*-locus homozygotes ($R_0 > R_1$).

Asymptotic Fate of General Local Perturbations

While equation (7) describes the change in frequency of a rare modifier allele over a single generation, evolutionary inference requires determination of whether rare alleles initiated in arbitrary genotypic configurations ultimately invade the population (Appendix 2). Beyond limiting cases (Appendix 3), the relative magnitudes of the direct and disequilibrium components of selection and their net effect on the asymptotic fate of rare alleles depends on the genetic structure of the population, including the nature of the association between mating type and modifiers of SI expression (δ).

Association between self-incompatibility enhancers and S-locus heterozygotes

We found that under a wide range of parameter assignments, enhancers of SI expression ($t_2 > t_0$) develop positive associations ($\delta > 0$) with *S*-locus heterozygosity (eq. A11). For viabilities of inbred offspring sufficiently low (eq. 11) to ensure that *S*-locus heterozygotes transmit modifier alleles at lower rates ($R_0 > R_1$), such associations with *S*-locus heterozygosity uniformly disfavor enhancers of SI. In particular, a greater than twofold difference in viability between inbred and outbred offspring ($1/2 > \sigma$) ensures that the disequilibrium effect promotes the invasion of suppressors of SI. Consequently, equation (9) represents a necessary condition for the evolutionary maintenance of SI: it ensures that the direct effect favor SI expression (dR_0/dt_0, $dR_1/dt_0 > 0$).

Incomplete Dominance

We denote the dominance of the introduced allele (M_1) by h: $t_1 = t_0 + h(t_2 - t_0)$, with $h = 0$ denoting complete recessivity, $h = 1/2$ additive expression, and $h = 1$ complete dominance. For simplicity, we restricted most of our analysis to modifier alleles that show complete dominance over the resident allele ($h = 1$, $t_1 = t_2$). To explore the evolutionary dynamics under other levels of dominance, we evaluated the condition for local stability within the full four-dimensional linearized system under a range of parameter assignments.

For a given set of values for the level of dominance, proportion of self pollen, number of segregating *S*-alleles, initial and new levels of SI expression, and level of reproductive compensation (h, s, n, t_0, t_2, and c), we determined the threshold viabilities of inbred offspring (σ) that permit or exclude the invasion of M_1. Our results indicate that incomplete dominance ($h < 1$) permits the maintenance of SI under slightly larger values of σ. This finding accords with that of an analysis of substitutions at the *S*-locus itself (Uyenoyama 1988b),

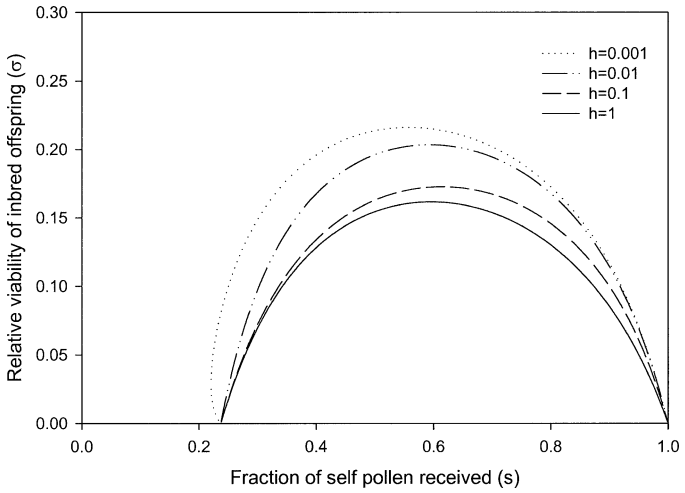

FIG. 1. Higher levels of dominance (h) of introduced modifier alleles reduce the maximum viability of inbred offspring that favors self-incompatibility (SI) expression ($t_0 = 0.9$, $n = 3$, $c = 0.9$). Rare modifier alleles that enhance SI expression increase only for parameter combinations that lie beneath the curves.

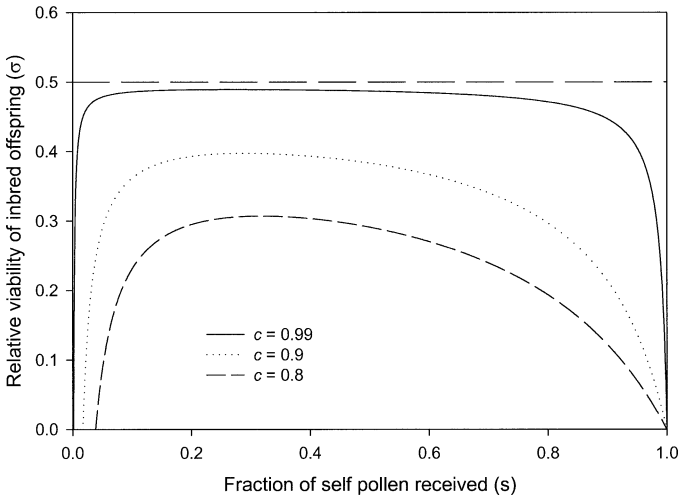

FIG. 2. Declines in the level of reproductive compensation (c) reduce the maximum viability of inbred offspring that favors dominant ($h = 1$) enhancers of self-incompatibility expression. Such enhancers increase when rare for parameter combinations corresponding to regions beneath the curves ($t_0 = 0.9$, $n = 15$). The horizontal dashed line corresponds to complete reproductive compensation ($c = 1$).

which indicated that lower levels of dominance in pistil expression tend to promote the invasion of active *S*-alleles into SC populations (see fig. 9 in Uyenoyama 1991). Even so, Figure 1 suggests that the thresholds for even small departures from complete recessivity ($h = 0$) correspond very closely to the bound for complete dominance ($h = 1$).

For most parameter sets, enhancers of SI expression invade only for values of σ below a single threshold value. However, multiple threshold values of σ can arise under low rates of selfing and levels of dominance of the introduced modifier allele. For example, Figure 1 indicates that in a narrow window of relatively low rates of receipt of self pollen (s), nearly completely recessive ($h = 0.001$) suppressors of SI increase when rare under both very low and higher relative inbred viabilities (σ), with enhancers favored in a small intervening range.

Incomplete Reproductive Compensation

The fate of a rare dominant modifier allele depends on the rates at which it is transmitted through *S*-locus homozygotes and heterozygotes (R_0 and R_1) and on relative *S*-locus heterozygosity among carriers and noncarriers (δ, u_1, and u_0). Under complete reproductive compensation ($c = 1$), these quantities depend only on whether the difference in viability between inbred and outbred offspring exceeds twofold ($1/2 > \sigma$; Appendix 3). Incomplete reproductive compensation ($c < 1$) introduces a dependence on the level of SI expression (t_0) as well.

Evolutionary costs

Figure 2 illustrates, for a range of parameter values, the regions in which enhancers of SI expression are favored or disfavored by the direct selection component (coefficient of q in eq. 7). For nearly complete reproductive compensation ($c \to 1$), the demarcation line between the two zones converges to a twofold difference in viability ($\sigma = 1/2$). Costs of SI expression increase with declining reproductive compensation (c). Furthermore, SI expression imposes greater restrictions on mating in populations with fewer segregating *S*-alleles (n; Fig. 3). Positive associations between enhancers of SI expression and *S*-locus heterozygotes, which transmit modifier alleles at lower rates under strong inbreeding depression (eq. 11), induce a small additional increase in the cost of SI expression (Fig. 4).

Evolutionary stability

Incomplete reproductive compensation ($c < 1$) can generate the novel phenomenon of evolutionarily stable intermediate levels of SI expression. Christiansen (1991) has re-

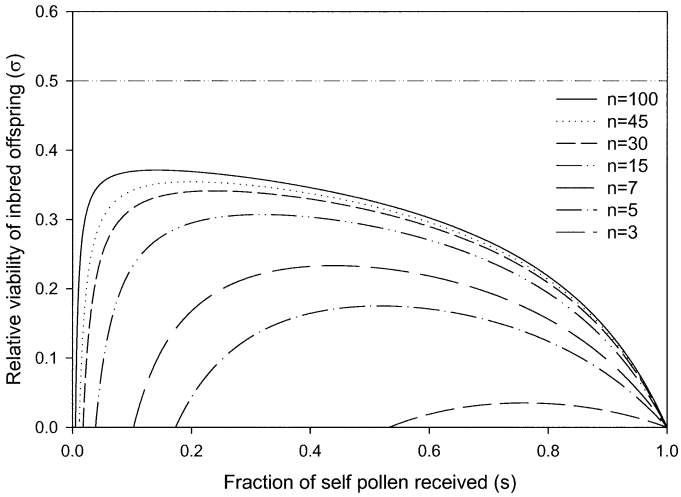

FIG. 3. Costs of self-incompatibility (SI) expression increase with declining numbers of segregating *S*-alleles (n). Dominant ($h = 1$) enhancers of SI expression invade populations only for viabilities of inbred offspring (σ) lying below the curves ($t_0 = 0.9$, $c = 0.8$).

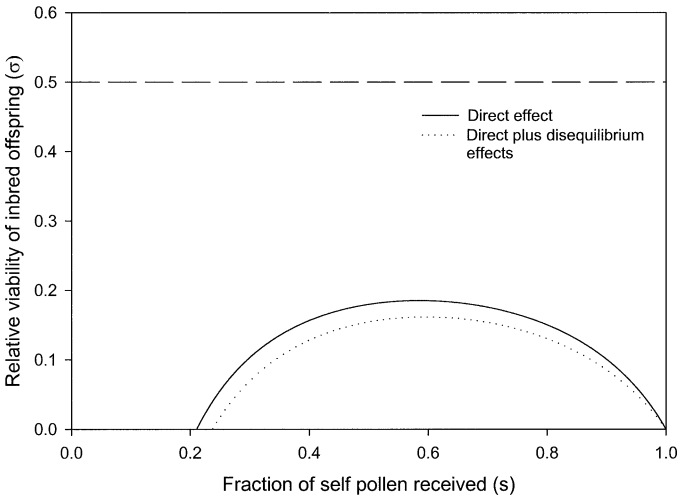

FIG. 4. Disequilibrium between the modifier locus and the *S*-locus causes a small increase in the cost of self-incompatibility (SI) expression. The lower curve depicts the maximum viability of inbred offspring (σ) that favors the invasion of dominant ($h = 1$) enhancers of SI, with other parameters as in Figure 1 (from eq. 7). The upper curve indicates the bound suggested by the direct effect (eq. 16) alone.

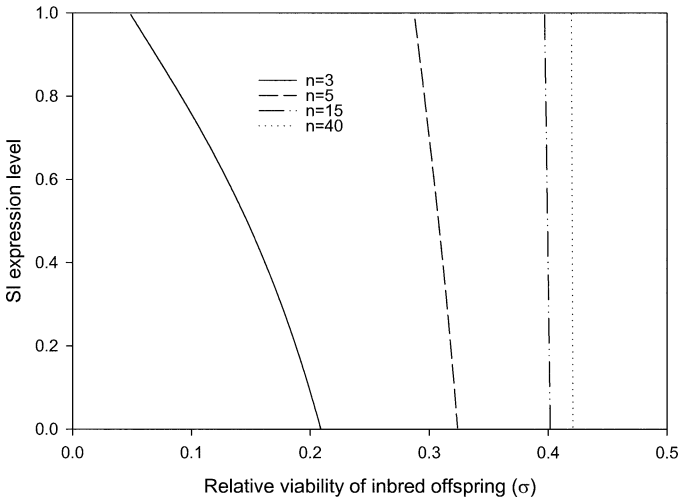

FIG. 5. Evolutionarily stable levels of self-incompatibility (SI) expression for a range of numbers of segregating *S*-alleles ($h = 0.5$, $c = 0.9$, $s = 0.3$). Enhancers of SI expression are favored only for sufficiently low viabilities of inbred offspring (σ values to the left of the curves). Points on the curves themselves correspond to evolutionarily stable levels of partial SI expression.

viewed various notions of evolutionary stability (ES). In particular, the trait value $\hat{t}$ represents a continuously stable strategy (CSS, Eshel and Motro 1981) if: (1) near the state of fixation of a modifier allele that determines $\hat{t}$, any modifier allele that determines a different level of expression fails to increase when rare; and (2) near the state of fixation of a modifier allele that determines $\hat{t} + \epsilon_0$, a modifier allele that determines $\hat{t} + \epsilon_1$ will increase when rare provided that

$$\epsilon_0(\epsilon_0 - \epsilon_1) > 0, \tag{12}$$

for ϵ_0 and ϵ_1 sufficiently close to zero. Condition (12) indicates that only alleles that initially bring the average level of expression in the population closer to $\hat{t}$ can invade. For genetically monomorphic populations, a CSS conforms most closely to an intuitive notion of the outcome of long-term evolution: such states are attracting (the average value of the trait in a population tends to converge to a CSS) and resistant to invasion (CSS values once attained tend to be preserved).

We identified candidate ES states and explored their evolutionary stability properties relative to modifier mutations of weak effect (small $t_0 - t_2$). For a number of parameter sets (h, c, n, s, σ), we determined values of the resident level of SI expression (t_0) at which the frequency of the rare modifier allele changes at less than geometric rates in the linearized recursion system ($\Delta q = 0$ in eq. 7). Such expression levels correspond to a superset of states that possess the first property of a CSS. We then tested the attractiveness and resistance to invasion of these candidates by iterating the full recursion system (Appendix 1). Our preliminary analysis indicates that most ES candidates do in fact correspond to CSSs: they are both attracting and resistant to invasion. Figure 5 shows examples of evolutionarily stable levels of partial SI expression for mutations of additive effect ($h = 1/2$); similar behavior arises under other dominance levels. In addition, under nearly complete recessivity ($h = 0.001$), some candidates appear to be anti-ESS states (Uyenoyama and Bengtsson 1982): attracting but susceptible to invasion.

DISCUSSION

We have explored the evolutionary dynamics of enhancers or suppressors of SI expression in populations subject to incomplete reproductive compensation. In the introductory section, we summarized experimental evidence of several genetic mechanisms for the modification of SI expression. We have addressed evolutionary changes at a modifier locus, unlinked to the *S*-locus, which determines the proportion of pollen tubes screened for compatibility. Our results indicate that incomplete reproductive compensation greatly increases the evolutionary costs of SI, particularly in populations with low *S*-allele diversity. Unexpectedly, it can also bring into existence evolutionarily stable levels of partial SI expression.

We first review the evolutionary costs of SI by comparing our results to those of other theoretical studies in which the genetic basis of the breakdown of SI has been explicitly represented. Incomplete reproductive compensation reduces the adaptiveness of SI as a mechanism of parental manipulation. We then describe a heuristic device (a phenotypic fitness model; Lloyd 1979) that recovers one of the two components of selection on modifiers of SI expression found in our genetic analysis of the evolutionary dynamics (eq. 7). Finally, we address whether incomplete SI expression may represent an evolutionarily stable state rather than a transient condition between full SI and full SC.

Self-Incompatibility as a Eugenic Strategy

Rejection of viable pollen can improve fertility only to the extent that future reproductive success compensates for any immediate loss of reproductive success. Under complete reproductive compensation ($c = 1$), pollen rejection in fact entails no immediate reduction in seed set; the adaptive value

of SI expression dependent only on the relative viability and relatedness of offspring. Under incomplete reproductive compensation ($c < 1$), the direct decline of immediate seed set with SI expression constitutes an evolutionary cost to deferred fertilization (Fig. 2).

By inhibiting fertilization by all self pollen but only some fraction of outcross pollen, SI can raise the proportion of seeds set by outcross pollen. Low numbers of segregating *S*-alleles (n) tend to disfavor enhancers of SI expression by increasing the fraction of outcross pollen rejected (Fig. 3). If nearly all pollen received is outcross pollen (s close to zero), pollen rejection reduces seed set while only slightly improving the production of outbred offspring. If nearly all pollen received is self pollen (s close to one), SI expression severely reduces seed set. Accordingly, intermediate levels of receipt of self pollen are most conducive to the maintenance of SI (Fig. 2).

Genetic Models of Self-Incompatibility Breakdown

Substitutions at the S-locus

Charlesworth and Charlesworth (1979) studied the breakdown of SI systems through the substitution of defective *S*-alleles. In contrast with the canonical formulation of GSI (Wright 1939), their model assumed complete absence of reproductive compensation. Uyenoyama (1988a,b) and Steinbachs and Holsinger (1999) derived conditions for the origin of SI from an SC state under full reproductive compensation.

Invasion of self-compatible populations by *S*-alleles with dominant expression in pistil occurs only under severe inbreeding depression:

$$\frac{1}{2} > \frac{1 - s}{3 - 2s} > \sigma \tag{13}$$

(Uyenoyama 1988a), for σ the relative viability of inbred offspring and s the proportion of self pollen received on stigmas. This expression illustrates that the cost of outcrossing incurred by alleles at the *S*-locus itself can considerably exceed twofold: by specifically rejecting pollen that express the same *S*-allele, SI expression causes the maternal and paternal complements to differ more than random. The maximal viability of inbred offspring that favors SI returns to twofold under recessive pistil expression (Charlesworth and Charlesworth 1979; Uyenoyama 1988b,c; Steinbachs and Holsinger 1999).

The classical twofold difference in relatedness between parents and offspring generated by outcrossing and selfing reflects random sampling of the allele received through outcross pollen. In contrast, *S*-allele-specific rejection of pollen causes the *S*-allele borne by compatible outcross pollen to differ more than randomly from the *S*-alleles of the maternal parent. The consequent reduction in parent-offspring relatedness with respect to the *S*-locus inflates the evolutionary costs of SI beyond twofold (Uyenoyama 1988b).

Substitutions at unlinked loci

Uyenoyama (1988c) explored Mather's (1943) proposal that SI originated through the recognition of alleles segregating at a proto-*S*-locus. In this evolutionary scenario, the ancestral *S*-locus initially served only as a switch gene, a highly polymorphic locus for which similarity between pistil and pollen genotype was indicative of genealogical relationship. Under complete reproductive compensation, a twofold difference in viability of inbred and outbred offspring is sufficient to favor the invasion of factors that permit such recognition (see eq. 16 in Uyenoyama 1988c). This result reflects the restoration of the twofold difference in parent-offspring relatedness with respect to modifiers unlinked to the *S*-locus. Like linkage ($r < 1/2$), incomplete reproductive compensation ($c < 1$) increases the evolutionary cost of SI beyond twofold (9).

A Phenotypic Fitness Analysis of Self-Incompatibility Breakdown

Lloyd's (1979) phenotypic fitness approach provides a heuristic device for the evolutionary analysis of trade-offs among ecological and genetic factors. Genetic evolution is implicit in this method, with genetic transmission subsumed under parent-offspring relatedness. It assumes that evolutionary trajectories converge to states that correspond to maxima of proposed fitness functions. These fitness functions comprise numbers of offspring produced through various modes of reproduction, weighted by parent-offspring relatedness. We find that the phenotypic fitness approach recovers the direct effect on modifiers of SI expression, but not the disequilibrium effect, which derives from evolved genetic associations between modifiers of SI expression and *S*-locus heterozygosity (eq. 7).

To study the evolution of self-fertilization, Lloyd (1992) proposed the phenotypic fitness function

$$w_i = x_i + 2\sigma y_i + p_i\frac{\bar{x}}{\bar{p}}, \tag{14}$$

in which w_i represents the fitness of phenotype i. It depends on the number of ovules produced by phenotype i that are fertilized by outcross pollen (x_i), the number fertilized by self pollen (y_i), and the relative viability of inbred offspring (σ). Reproductive success of phenotype i through pollen depends on its contribution to the outcross pollen pool (p_i) relative to the average pollen contribution ($\bar{p}$) and the average number of outcrossed ovules ($\bar{x}$). The factor of 2 against y_i reflects the twofold increase in parent-offspring relatedness under selfing. Outbreeder phenotype 0, which sets fewer seeds by self pollen ($y_1 > y_0$), has higher phenotypic fitness if

$$\left(\frac{x_0 - x_1}{y_1 - y_0}\right) + \left(\frac{p_0 - p_1}{y_1 - y_0}\right)\frac{\bar{x}}{\bar{p}} > 2\sigma \tag{15}$$

(compare eq. 1b of Lloyd 1992). The second term on the left represents the effects of pollen discounting (Holsinger et al. 1984), the reduction in contribution to the outcross pollen pool that an increase in seed set by self pollen entails. Analogously, the other term on the left represents seed discounting (Lloyd 1992), correlated changes in the numbers of seeds set by outcross and self pollen. In particular, seed discounting is absent if the two phenotypes set identical total numbers of seeds ($x_0 + y_0 = x_1 + y_1$), as expected in the absence of pollen limitation. Candidates for evolutionarily stable mating

systems satisfy condition (15) with the inequality replaced by an equality.

Our analysis addresses the evolution of modifiers of SI expression in the absence of pollen discounting ($p_0 = p_1$). Of the two components of selection arising in the evolutionary modification of SI expression (7), the direct effect, which reflects trade-offs among parent-offspring relatedness, offspring viability, and seed set, resembles a phenotypic fitness function. This component favors enhancers of SI if

$$nu_0\frac{dR_0}{dt_0} + \binom{n}{2}u_1\frac{dR_1}{dt_0} > 0, \tag{16}$$

for R_0 and R_1 (eq. 8) measures of transmission of modifier alleles through *S*-locus homozygotes and heterozygotes, respectively, and t_0 the current prevailing level of SI expression. To recast this component of selection in terms of phenotypic fitnesses (14), we represent the transmission of modifier alleles through *S*-locus homozygotes by

$$R_0 = (x_0 + 2\sigma y_0)/2, \tag{17}$$

for x_0 and y_0 seed set by outcross and self pollen:

$$x_0 = \frac{(1 - s)[(1 - t_0)/n + (1 - 1/n)]}{d_0} \quad \text{and} \tag{18a}$$

$$y_0 = \frac{s(1 - t_0)}{d_0}. \tag{18b}$$

Enhanced SI expression promotes transmission of modifier alleles through *S*-locus homozygotes if

$$\frac{dR_0}{dt_0} = \left(\frac{dx_0}{dt_0} + 2\sigma\frac{dy_0}{dt_0}\right)\Big/ 2 > 0. \tag{19}$$

Because greater SI expression reduces seed set through self pollen ($dy_0/dt_0 < 0$), this condition entails

$$2\sigma < \frac{dx_0/dt_0}{-dy_0/dt_0} = \pi_0, \tag{20}$$

for π_0 the seed discounting component of condition (15). From equations (18a, b), we obtain

$$\pi_0 = \frac{(1 - s)[cs(1 - 1/n) - (1 - c)/n]}{s[c(1 - s)(1 - 1/n) + (1 - c)]}. \tag{21}$$

Incorporating similar expressions for rates of transmission of modifier alleles through *S*-locus heterozygotes (R_1), we obtain an alternative representation of (16):

$$nu_0\pi_0 + \binom{n}{2}u_1\pi_1 > 2\sigma, \tag{22}$$

(compare condition 15), for

$$\pi_1 = \frac{(1 - s)[cs(1 - 2/n) - (1 - c)2/n]}{s[c(1 - s)(1 - 2/n) + (1 - c)]} \leq \pi_0. \tag{23}$$

Both π_0 and π_1 attain their maximum value of unity under complete reproductive compensation ($c = 1$), which yields the twofold cost of outcrossing expected in the absence of pollen discounting (see eq. 20). Incomplete reproductive compensation ($c < 1$) induces higher levels of seed discounting, which tends to discourage SI expression; this effect is greater in *S*-locus heterozygotes than homozygotes.

Mixed Mating Systems

Evolutionary stability

In addition to substantially reducing the maximal viability of inbred offspring that permits the maintenance of SI, incomplete reproductive compensation gives rise to evolutionarily stable levels of partial expression of SI (Fig. 5). These states exist only in a narrow transition zone between complete expression (low σ, to the left of the curves in Fig. 5) and complete suppression (higher σ). The curves converge rapidly to virtual step functions as the number of segregating *S*-alleles (n) or level of reproductive compensation (c) increase. Even so, the very existence of evolutionarily stable mixed mating systems was not anticipated.

Evolutionarily stable levels of partial SI expression reflect exact cancellation between the disequilibrium (eq. 10) and direct (eq. 16) effects of SI on the transmission of modifier alleles (see eq. 7). Consideration of phenotypic fitnesses (direct effect alone) would suggest that candidates for evolutionarily stable states satisfy

$$nu_0(\pi_0 - 2\sigma) + \binom{n}{2}u_1(\pi_1 - 2\sigma) = 0 \tag{24}$$

(from eq. 22). This condition requires that inbreeding depression be sufficiently intense to compensate for seed discounting in *S*-locus homozygotes but not in heterozygotes:

$$\pi_0 > 2\sigma > \pi_1. \tag{25}$$

Our dynamical analysis, which explicitly addresses the genetic evolution of modifiers of SI expression, indicates that enhancers of SI evolve positive genetic associations with *S*-locus heterozygosity (eq. A11). Under incomplete reproductive compensation, this disequilibrium effect tends to oppose SI because *S*-locus heterozygotes reject more *S*-alleles than do homozygotes (eq. 10). Evolutionarily stable states in fact correspond to a balance between a positive association between phenotypic fitness and expression of SI (16) and the negative consequences of the genetic association (10). Such effects arise even in the absence of linkage between the modifier of SI and the *S*-locus, the condition least conducive to the generation of disequilibrium. While disequilibrium between the modifier and the *S*-locus generates only a small increase in the evolutionary costs of SI in our study (Fig. 4), its influence likely grows as linkage tightens.

Coevolution of components of self-incompatibility systems

Although our analysis treats the number of segregating *S*-alleles (n) as a parameter, it undoubtedly coevolves with the level of SI expression. Because the costs of SI grow as the number of *S*-alleles declines (Fig. 3), reductions in the number of *S*-alleles with SI expression would intensify any initial selective disadvantage of SI for a given level of inbreeding depression. Similarly, any initial selective advantage of enhanced SI expression would be reinforced by consequent increases in *S*-allele number.

Changes in the intensity of inbreeding depression may modulate this pressure toward full expression or full suppression of SI. We propose that coevolution on comparable time scales of inbreeding depression and the mating system

may admit evolutionarily stable levels of partial SI expression under less restrictive conditions. Genetic associations between factors contributing to inbreeding depression and modifiers of SI expression likely influence the minimal intensity of inbreeding depression required for the maintenance of SI. Previous studies of the evolutionary modification of selfing rate suggest that such associations can decrease (Uyenoyama and Waller 1991a) as well as increase (Uyenoyama and Waller 1991b) the evolutionary costs of outbreeding. In particular, SI may be maintained even under less than twofold levels of inbreeding depression (Uyenoyama 1991).

Evolutionary Significance of Incomplete Self-Incompatibility Expression

Expression of SI may incur lower evolutionary costs than suggested by our analysis in perennial species, for which reproductive compensation may be realized over multiple breeding seasons (Goodwillie 1999; Larson and Barrett 2000). Allocating more reproductive resources to seasons that offer better opportunities for outcrossing may bear evolutionary advantages similar to those deriving from within-season serial adjustment (Lloyd 1980) of maternal resources among successive developmental stages. In particular, the abscission of self-pollinated flowers in *Phormium tenax* (Becerra and Lloyd 1992) and the positive association between rates of fruit abortion and SI expression in *Campanula rapunculoides* (Vogler and Stephenson 2001) may represent adaptations rather than unconditionally deleterious by-products of self-fertilization or expression of SI. Reproductive assurance (Lloyd 1979) reflects the advantage of producing even low quality offspring over no offspring at all. In contrast, reproductive compensation across multiple seasons would confer benefits even under full SI expression.

Phylogenetically independent contrasts provide some support for the view that having multiple mating opportunities may alleviate pollen limitation (Larson and Barrett 2000). Woody species (all polycarpic) showed significantly greater improvement in fruit set under pollen supplementation than did herbaceous species (polycarpic and monocarpic); however, the contrast between polycarpic and monocarpic species under restriction to self-incompatible herbs indicated nonsignificant differences.

An intriguing question concerns whether incomplete expression of SI represents an evolutionarily stable state rather than a transient condition between complete expression and complete breakdown of SI (Levin 1996; Stephenson et al. 2000). In particular, the history of duplication or polyploidization in *C. rapunculoides* suggested by allozyme segregation patterns (Vogler and Stephenson 2001) may indicate that partial breakdown of SI in this species merely represents a by-product of the duplication of part of the *S*-locus. Alternatively, attenuation of SI expression may directly confer selective advantages in this species, which commonly resides in small, isolated populations (Vogler et al. 1999), conditions shown to be associated with low cross-compatibility (Levin 1989; Byers and Meagher 1992; Goodell et al. 1997). Indeed, the existence of many possible points of control of SI expression (McClure et al. 2000; Cruz-García et al. 2003) and the demonstrated heritability of age-dependent breakdown in this species (Good-Ávila and Stephenson 2002) suggest that partial SI expression may represent an adaptation and not merely a transient condition.

Acknowledgments

This manuscript is in appreciation of the contributions of David G. Lloyd. We thank S. Good-Ávila and D. Wolf for comments and suggestions and an anonymous reviewer for pointing out a significant typographical error. A Consejo Nacional de Ciencia y Tecnología (Mexico) graduate fellowship to MVM and U.S. Public Health Service grant GM 37841 to MKU provided support for this study.

Literature Cited

Baker, H. G. 1955. Self-compatibility and establishment after ''long-distance'' dispersal. Evolution 9:347–349.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. Heredity 2:349–368.

Becerra, J. X., and D. G. Lloyd. 1992. Competition dependent abscission of self-polinated flowers of *Phormium tenax*: a second action of self-incompatibility at the whole flower level. Evolution 46:90–110.

Bertin, R. I., and M. Sullivan. 1988. Pollen interference and cryptic self-fertility in *Campsis radicans*. Amer. J. Bot. 75:1140–1147.

Bixby, P. J., and D. Levin. 1996. Response to selection on autogamy in *Phlox*. Evolution 50:892–899.

Burd, M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. Bot. Rev. 60: 83–139.

Byers, D. L., and T. R. Meagher. 1992. Mate availability in small populations of plant species with homomorphic sporophytic self-incompatibility. Heredity 68:353–359.

Charlesworth, D., and B. Charlesworth. 1979. The evolution and breakdown of *S*-allele systems. Heredity 43:41–55.

Christiansen, F. B. 1991. On conditions for evolutionary stability for a continuously varying character. Am. Nat. 138:37–50.

Conner, J. A., T. Tantikanjana, J. C. Stein, M. K. Kandasamy, J. B. Nasrallah, and M. E. Nasrallah. 1997. Transgene-induced silencing of *S*-locus genes and related genes in *Brassica*. Plant J. 11:809–823.

Cruz-García, F., C. N. Hancock, and B. McClure. 2003. S-RNase complexes and pollen rejection. J. Exp. Bot. 54:123–130.

de Nettancourt, D. 1977. Incompatibility in angiosperms. Springer, Berlin.

Eshel, I., and U. Motro. 1981. Kin selection and strong evolutionary stability of mutual help. Theor. Pop. Biol. 19:410–433.

Finney, D. J. 1952. The equilibrium of a self-incompatible polymorphic species. Genetica 26:33–64.

Fisher, R. A. 1930. The genetical theory of natural selection. Oxford Univ. Press, Oxford, U.K.

Fishman, L., and R. Wyatt. 1999. Pollinator-mediated competition, reproductive character displacement, and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). Evolution 53: 1723–1733.

Galen, C., T. Gregory, and L. F. Galloway. 1989. Costs of self-pollination in a self-incompatible plant. Amer. J. Bot. 76: 1675–1680.

Gantmacher, F. R. 1959. The theory of matrices. Chelsea, New York.

Golz, J. F., V. Su, A. E. Clarke, and E. Newbigin. 1999. A molecular description of mutations affecting the pollen component of the *Nicotiana alata S*-locus. Genetics 152:1123–1135.

Golz, J. F., H. Y. Oh, M. Kusaba, and E. Newbigin. 2001. Genetic analysis of nicotiana pollen-part mutants is consistent with the presence of an *S*-ribonuclease inhibitor at the *S*-locus. Genetics 98:15372–15376.

Good-Ávila, S. V., and A. G. Stephenson. 2002. The inheritance of modifiers conferring self-fertility in the partially self-incom-

patible perennial, *Campanula rapunculoides* L. (Campanulaceae). Evolution 56:263–272.
Goodell, K., D. R. Elam, J. D. Nason, and N. C. Ellstrand. 1997. Gene flow among small populations of a self-incompatible plant: an interaction between demography and genetics. Amer. J. Bot. 84:1362–1371.
Goodwillie, C. 1999. Multiple origins of self-incompatibility in *Linanthus* section *Leptosiphon* (Polemoniaceae): phylogenetic evidence from internal-transcribed-spacer sequence data. Evolution 53:1387–1395.
Holsinger, K. E., M. W. Feldman, and F. B. Christiansen. 1984. The evolution of self-fertilization in plants: a population genetic model. Am. Nat. 124:446–453.
Ikeda, S., J. B. Nasrallah, R. Dixit, S. Preiss, and M. Nasrallah. 1997. An aquaporine-like gene required for the *Brassica* self-incompatibility response. Science 276:1564–1566.
Koenig, W. D., and M. V. Ashley. 2003. Is pollen limited? The answer is blowin' in the wind. Trends Ecol. Evol. 18:157–159.
Larson, B. M. H., and S. C. H. Barrett. 2000. A comparative analysis of pollen limitation in flowering plants. Biol. J. Linn. Soc. 69: 503–520.
Lee, H. S., S. S. Huang, and T.-h. Kao. 1994. *S*-proteins control rejection of incompatible pollen in *Petunia inflata*. Nature 367: 550–563.
Levin, D. A. 1989. Proximity-dependence cross-compatibility in *Phlox*. Evolution 43:1114–1116.
———. 1996. The evolutionary significance of pseudo-self-fertility. Am. Nat. 148:321–332.
Lewis, D. 1943. Physiology of incompatibility in plants. III. Autoployploids. J. Genetics 45:171–185.
Lloyd, D. G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. Am. Nat. 113:67–79.
———. 1980. Sexual strategies in plants. I. An hypothesis of serial adjustment of maternal investment during one reproductive session. New Phytol. 86:69–79.
———. 1987. Allocations to pollen, seeds and pollination mechanisms in self-fertilizing plants. Func. Ecolo. 1:83–89.
———. 1992. Self-fertilization and cross-fertilization in plants. II. The selection of self-fertilization. Int. J. Plant Sci. 153:370–380.
Mather, K. 1943. Specific differences in *Petunia*. J. Genetics 45: 215–235.
Maurice, S., and T. H. Fleming. 1995. The effect of pollen limitation on plant reproductive systems and the maintenance of sexual polymorphisms. Oikos 74:55–60.
McClure, B., B. Mou, S. Canevascini, and R. Bernatzky. 1999. A small asparagine-rich protein required for *S*-allele-specific pollen rejection in *Nicotiana*. Proc. Natl. Acad. Sci. USA 96: 13548–13553.
McClure, B. A., F. Cruz-García, B. Beecher, and W. Sulaman. 2000. Factors affecting inter- and intra-specific pollen rejection in *Nicotiana*. Ann. Bot. (Lond) 85:113–123.
Morse, D. H. 1994. The role of self-pollen in the female reproductive success of common milkweed (*Asclepias syriaca*: Asclepiadaceae). Amer. J. Bot. 81:322–330.
Murfett, J., E. C. Cornish, P. R. Ebert, I. Bonig, B. A. McClure, and A. E. Clarke. 1992. Expression of a sef-incompatibility glycoprotein (*S*2-ribonuclease) from *Nicotiana alata* in transgenic *Nicotiana tabacum*. Plant Cell 4:1063–1074.
Murfett, J., T. L. Atherton, B. Mou, C. S. Gasser, and B. A. McClure. 1994. *S-RNase* expressed in transgenic *Nicotiana* causes *S*-allele-specific pollen rejection. Nature 367:563–566.
Nasrallah, M., M. K. Kandasamy, and J. B. Nasrallah. 1992. A genetically defined *trans*-acting locus regulates *S*-locus function in *Brassica*. Plant J. 2:497–506.
Ockendon, D. J., and L. Currah. 1977. Self-pollen reduces the number of cross-pollen tubes in the styles of *Brassica oleracea* L. New Phytol. 78:675–680.
Olivieri, I., D. Couvet, and M. Slatkin. 1994. Allocation of reproductive effort in perennial plants under pollen limitation. Am. Nat. 144:373–394.
Ramsey, M., and G. Vaughton. 2000. Pollen quality limits seed set in *Burchardia umbellata* (Colchicaceae). Amer. J. Bot. 87: 845–852.
Reinartz, J. A., and D. H. Les. 1994. Bottleneck-induced dissolution of self-incompatibility and breeding. Amer. J. Bot. 81:446–455.
Rick, C. M. 1986. Reproductive isolation in the *Lycopersicon peruvianum* complex. Pp. 477–495 *in* W. G. D'arcy, ed. Solanaceae: biology and systematics. Columbia Univ. Press, New York.
Royo, J., C. Kunz, Y. Kowyama, M. Anderson, A. E. Clark, and E. Newbigin. 1994. Loss of a histidine residue at the active site of *S*-locus ribonuclease is associated with self-compatibility in *Lycopersicon peruvianum*. Proc. Natl. Acad. Sci. USA 91: 6511–6514.
Sakai, S. 1995. Evolutionarily stable selfing rates of hermaphroditic plant in competing and delayed selfing modes with allocation to attractive structures. Evolution 49:557–564.
Satake, A., and Y. Iwasa. 2002. Spatially limited pollen exchange and a long-range synchronization of trees. Ecology 83: 993–1005.
Shore, J. S., and S. C. H. Barrett. 1984. The effect of pollination intensity and incompatible pollen on seed set in *Turnera ulmifolia* (Turneraceae). Can. J. Bot. 62:1298–1303.
Stebbins, G. L. 1955. Variation and evolution in plants. Columbia Univ. Press, New York.
Steinbachs, J. E., and K. E. Holsinger. 1999. Pollen transfer dynamics and the evolution of gametophytic self-incompatibility. J. Evol Biol. 12:770–778.
Stephenson, A. G., S. V. Good, and D. W. Vogler. 2000. Interrelationships among inbreeding depression, plasticity in the self-incompatibility system, and the breeding system of *Campanula rapunculoides* L. (Campanulaceae). Ann. Bot. (Lond) 85(Suppl. A):211–219.
Stephenson, A. G., S. E. Travers, J. I. Mena-Ali, and J. A. Winsor. 2003. Pollen performance before and during the autotrophic-heterotrophic transition of pollen tube growth. Phil. Trans. R. Soc. Lond. B 358:1009–1018.
Takasaki, T., K. Hatakeyama, G. Suzuki, M. Watanabe, A. Isogai, and K. Hinata. 2000. The *S* receptor kinase determines self-incompatibility in *Brassica* stigma. Nature 403:913–916.
Tsukamoto, T., T. Ando, H. Kokubun, H. Watanabe, T. Sato, M. Masada, E. Marchesi, and T.-h. Kao. 2003. Breakdown of self-incompatibility in a natural population of *Petunia axillaris* caused by a modifier locus that suppresses the expression of an *S*-RNase gene. Sex. Plant Reprod. 15:255–263.
Uyenoyama, M. K. 1988a. On the evolution of genetic incompatibility systems. II. Initial increase of strong gametophytic self-incompatibility under partial selfing and half-sib mating. Am. Nat. 131:700–722.
———. 1988b. On the evolution of genetic incompatibility systems. III. Introduction of weak gametophytic self-incompatibility under partial inbreeding. Theor. Pop. Biol. 34:47–91.
———. 1988c. On the evolution of genetic incompatibility systems. IV. Modification of response to an existing antigen polymorphism under partial selfing. Theor. Pop. Biol. 34:347–377.
———. 1991. On the evolution of genetic incompatibility systems. VI. A three-locus modifier model for the origin of gametophytic self-incompatibility. Genetics 128:453–459.
Uyenoyama, M. K., and B. O. Bengtsson. 1982. Towards a genetic theory for the evolution of the sex ratio. III. Parental and sibling control of brood investment ratio under partial sib mating. Theor. Pop. Biol. 22:43–68.
Uyenoyama, M. K., and D. M. Waller. 1991a. Coevolution of self-fertilization and inbreeding depression. II. Symmetric overdominance in viability. Theor. Pop. Biol. 40:47–77.
———. 1991b. Coevolution of self-fertilization and inbreeding depression. III. Homozygous lethal mutations at multiple loci. Theor. Pop. Biol. 40:173–210.
Vekemans, X., M. H. Schierup, and F. B. Christiansen. 1998. Mate availability and fecundity selection in multi-allelic self-incompatibility systems in plants. Evolution 52:19–29.
Vogler, D. W., and A. G. Stephenson. 2001. The potential for mixed mating in a self-incompatible plant. Int. J. Plant Sci. 162: 801–805.
Vogler, D. W., K. Filmore, and A. G. Stephenson. 1999. Inbreeding depression in *Campanula ranunculoides* L. I. A comparison of

inbreeding depression in plants derived from strong and weak self-incompatibility phenotypes. J. Evol Biol. 12:483–494.
Wilcock, C., and R. Neiland. 2002. Pollination failure in plants: why it happens and when it matters. Trends Plant Sci. 7:270–277.
Wright, S. 1939. The distribution of self-sterility alleles in populations. Genetics 24:538–552.

Corresponding Editor: T. Day

Appendix 1

Recursion System

Recursions in gene and genotypic frequencies correspond to:

$$Tu_0' = s\sigma[(1-t_0)[u_0^* + (n-1)u_1^*/4] + (1-t_1)\{v_0^* + (n-1)v_1^*[r^2 + (1-r)^2]/2\}/4] + (1-s)p\{(1-t_0)[u_0^* + (n-1)u_1^*/2] + (1-t_1)[v_0^* + (n-1)v_1^*/2]/2\}/n, \quad \text{(A1)}$$

$$Tu_1' = s\sigma[(1-t_0)u_1^* + (1-t_1)v_1^* r(1-r)]/2 + (1-s)2p[u_0^* + v_0^*/2 + (n-2)(u_1^* + v_1^*/2)/2 + (1-t_0)u_1^*/2 + (1-t_1)v_1^*/4]/n, \quad \text{(A2)}$$

$$Tv_0' = s\sigma(1-t_1)[v_0^* + r(1-r)(n-1)v_1^*]/2 + (1-s)p\{(1-t_2)[w_0^* + (n-1)w_1^*/2] + (1-t_1)[v_0^* + (n-1)v_1^*/2]/2\}/n + (1-s)q\{(1-t_0)[u_0^* + (n-1)u_1^*/2] + (1-t_1)[v_0^* + (n-1)v_1^*/2]/2\}/n, \quad \text{(A3)}$$

$$Tv_1' = s\sigma(1-t_1)v_1^*[r^2 + (1-r)^2]/2 + (1-s)2p[w_0^* + v_0^*/2 + (n-2)(w_1^* + v_1^*/2)/2 + (1-t_2)w_1^*/2 + (1-t_1)v_1^*/4]/n + (1-s)2q[u_0^* + v_0^*/2 + (n-2)(u_1^* + v_1^*/2)/2 + (1-t_0)u_1^*/2 + (1-t_1)v_1^*/4]/n, \quad \text{(A4)}$$

$$Tw_0' = s\sigma[(1-t_2)[w_0^* + (n-1)w_1^*/4] + (1-t_1)\{v_0^* + (n-1)v_1^*[r^2 + (1-r)^2]/2\}/4] + (1-s)q\{(1-t_2)[w_0^* + (n-1)w_1^*/2] + (1-t_1)[v_0^* + (n-1)v_1^*/2]/2\}/n, \quad \text{and} \quad \text{(A5)}$$

$$Tw_1' = s\sigma[(1-t_2)w_1^* + (1-t_1)v_1^* r(1-r)]/2 + (1-s)2q[w_0^* + v_0^*/2 + (n-2)(w_1^* + v_1^*/2)/2 + (1-t_2)w_1^*/2 + (1-t_1)v_1^*/4]/n, \quad \text{(A6)}$$

in which T ensures that the genotypic frequencies sum to unity:

$$nT(u_0' + v_0' + w_0') + \binom{n}{2}T(u_1' + v_1' + w_1') = T \quad \text{(A7)}$$

(compare eq. 2). Variables v_0^* and w_0^* take a form analogous to equation (3a), with the substitution of the appropriate SI expression level (t_1 or t_2), and v_1^* and w_1^* are analogous to equation (3b).

For very low frequencies of the modifier allele M_1, terms of the second order or smaller in the frequencies of gametes or genotypes that carry this allele are negligible. In this linearized system of recursions, T, u_0, and u_1 take their values at equilibrium, prior to the introduction of M_1 (from eq. 2, with $u_0' = u_0$ and $u_1' = u_1$).

Appendix 2

Local Stability Analysis

We determined the condition for the initial increase of a dominant modifier allele (M_1) introduced, in arbitrarily low frequency at a locus unlinked to the *S*-locus, into the equilibrium resident population ($\hat{u}_0$, $\hat{u}_1$). Ignoring terms of second order or smaller in the frequencies of carriers of M_1 produces a linear approximation of the evolutionary dynamics:

$$\begin{pmatrix} q' \\ \delta' \end{pmatrix} = \boldsymbol{M}\begin{pmatrix} q \\ \delta \end{pmatrix}. \quad \text{(A8)}$$

Because $\boldsymbol{M}$ is nonnegative, a necessary and sufficient condition for local stability (exclusion of M_1) is that the determinants of all principal minors of $[\boldsymbol{I} - \boldsymbol{M}]$ be positive (see Gantmacher 1959, p. 71). Our assumption of weak effect of the modifier (t_2 close to t_0) implies, for irreducible $\boldsymbol{M}$, that positivity of the characteristic equation of the linearized transformation evaluated at one is sufficient for local stability:

$$\mathrm{Det}[\boldsymbol{I} - \boldsymbol{M}] > 0, \quad \text{(A9)}$$

for Det the determinant. We verified the sufficiency of this criterion for biologically relevant cases associated with reducible matrices ($c = 0$, $c = 1$, $s = 1$).

We addressed the relationship between the ultimate fate of a rare modifier allele introduced in arbitrary genotypic configuration (A9) and the direction of change in its frequency over a single generation (eq. 7). For rare modifier alleles of sufficiently weak effect (small differences among t_0, t_1, and t_2), the direction of gene frequency change over a single generation from a particular genotypic configuration does in fact indicate asymptotic local stability. This configuration ($\tilde{q}$, $\tilde{\delta}$) is determined from the second row of

$$[\boldsymbol{M} - \boldsymbol{I}]\begin{pmatrix} \tilde{q} \\ \tilde{\delta} \end{pmatrix} = \begin{pmatrix} \Delta q \\ 0 \end{pmatrix} \quad \text{(A10)}$$

(Uyenoyama 1991). Numerical evaluation of this genotypic configuration under a wide range of parameter assignments indicates that modifier alleles that enhance SI expression develop positive associations with *S*-locus heterozygosity:

$$\tilde{\delta} \propto -\tilde{q}(t_0 - t_2). \quad \text{(A11)}$$

Appendix 3

Limiting Cases

Assignment of some parameters to the limits of their valid ranges can generate a reducible local stability matrix. In such cases, positivity of equation (7) remains a necessary condition for local stability, but it may not be sufficient. Here we confirm sufficiency for biologically relevant cases of this kind.

Under complete reproductive compensation ($c = 1$), equation (8) indicates that SI expression increases reproductive success in *S*-locus homozygotes and heterozygotes only if inbreeding reduces offspring viability by twofold or more:

$$\frac{dR_0}{dt_0}, \frac{dR_1}{dt_0} \propto s(1-s)(1-2\sigma). \quad \text{(A12)}$$

Viabilities in this range also ensure that *S*-locus homozygotes transmit modifier alleles at higher rates:

$$R_0 - R_1 \propto s(1-s)t_0(1-t_0)(1-2\sigma). \quad \text{(A13)}$$

These expressions indicate that under greater than twofold differences in viability between inbred and outbred offspring ($1/2 > \sigma$), the direct effect (A12) tends to favor and the disequilibrium effect (A13) to disfavor enhancers of SI expression. Evaluation of equation (7) at ($\tilde{q}$, $\tilde{\delta}$) indicates that the direct effect prevails, recovering previous results (Uyenoyama 1988c).

Under exclusive receipt of self pollen ($s = 1$), homozygotes and heterozygotes transmit modifier alleles at equal rates ($R_0 = R_1$, from

eq. 10) and increased SI expression uniformly reduces transmission (dR_0/dt_0, $dR_1/dt_0 < 0$). From (7),

$$-T\Delta q \propto -(t_0 - t_2), \tag{A14}$$

which indicates that SI expression is uniformly disfavored in the absence of outcross pollen.

Expression of SI is also uniformly disfavored in the absence of reproductive compensation ($c = 0$). Rejection of incompatible pollen only consigns to lethality zygotes that would have derived from those pollen. Irrespective of the relative viability of inbred offspring (σ), SI expression uniformly reduces offspring production (dR_0/dt_0, $dR_1/dt_0 < 0$, from eq. 9). Furthermore, *S*-locus heterozygotes, with which enhancers of SI expression are associated (eq. A11), transmit modifier alleles at lower rates than do homozygotes ($R_0 - R_1 > 0$, from eq. 10) because they reject a larger proportion of pollen. Consequently, both the direct and disequilibrium components of the one-generation change in modifier allele frequency (7) disfavor enhancers of SI expression.